Amendments to the Specification:

Replace the paragraph beginning at page 1, line 5, with the following amended paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 371 of PCT Application No. PCT/US2004/019229, filed June 10, 2004, now pending, which claims priority from U.S. Provisional Application No. 60/478,076, filed June 12, 2003, each of which is incorporated by reference herein in their entireties. This application also claims priority from U.S. Provisional Application No. 60/638,294, filed December 22, 2004, which is incorporated by reference herein in its entirety.

Replace the section beginning at page 3, line 29, and ending at page 6, line 26, with the following amended section:

A method for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising administering to said cell at least one double-stranded RNA effector molecule, preferably 2, 3, 4, 5, 6, or more double-stranded RNA effector molecules, each double-stranded RNA effector molecule comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:49; wherein U is substituted for T. In a preferred method, three or four dsRNA effector molecules, each comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:23, and SEQ ID NO:49; wherein U is substituted for T; are administered to an in vivo mammalian cell. The double-stranded RNA effector molecules may be prepared exogenously and administered into a mammalian cell or expressed intracellularly in a mammalian cell from a double-stranded RNA expression vector, i.e., an expression vector engineered to express a dsRNA effector molecule in a mammalian cell. In a preferred method, at least three or four dsRNA effector molecules, each comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:23, and

SEQ ID NO:49; wherein U is substituted for T; are encoded in a dsRNA expression vector which is administered to an in vivo mammalian cell.

A composition for inhibiting the expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising at least one, preferably 2, 3, 4, 5, 6 or more double-stranded RNA effector molecules, each double-stranded RNA effector molecule comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:49; wherein U is substituted for T. In a preferred composition, at least three or four dsRNA effector molecules are included, each comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:23, and SEQ ID NO:49; wherein U is substituted for T. The double-stranded RNA effector molecules may be prepared exogenogenously and the composition comprising two, three, four, five, six, or more dsRNA effector molecules administered into a mammalian cell, or the composition may comprise one or more dsRNA expression constructs capable of expressing in a mammalian cell two, three, four, five, six or more of said dsRNA effector molecules. In a preferred composition, three or four dsRNA effector molecules, each comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:23, and SEQ ID NO:49; wherein U is substituted for T, are encoded in a dsRNA expression vector.

A method for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising administering to said cell at least two, preferably 3, 4, 5, 6 or more, double-stranded RNA effector molecules, each double-stranded RNA effector molecule comprising: (a) a sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, and SEQ ID NO:62; (b) the reverse complement of said selected sequence; and (c) optionally, a sequence linking sequences (a) and (b); wherein U is substituted for T. In a preferred method, said dsRNA effector molecules will comprise 3 or 4 sequences selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO:59, and SEQ ID NO:62; wherein U is substituted for T. The double-stranded RNA effector molecules may be stem-loop or hairpin structures and/or duplex double-stranded RNA molecules. The double-stranded RNA effector molecules may be prepared exogenogenously and the two, three, four, five, six, or more dsRNA effector molecules administered into a mammalian cell, or one or more

dsRNA expression constructs capable of expressing in a mammalian cell two, three, four, five, six or more of said dsRNA effector molecules may be administered.

A composition for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising at least two double-stranded RNA effector molecules, each double-stranded RNA effector molecule comprising: (a) a sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, and SEQ ID NO:62; (b) the reverse complement of said selected sequence; and (c) optionally, a sequence linking sequences (a) and (b); wherein U is substituted for T. In a preferred composition, three or four of said dsRNA effector molecules will be included, or encoded in an expression vector, comprising sequences selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO:59, and SEQ ID NO:62; wherein U is substituted for T. The double-stranded RNA effector molecules may be prepared exogenously and the composition will comprise two, three, four, five, six, or more of said dsRNA effector molecules for administration into a in vivo mammalian cell, or the composition may comprise one or more dsRNA expression constructs capable of expressing in a mammalian cell two, three, four, five, six or more of said dsRNA effector molecules.

In another aspect the invention relates to methods and compositions for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising administering to said cell at least two, preferably 3, 4, 5, 6 or more, double-stranded RNA effector molecules, each double-stranded RNA effector molecule comprising: (a) a sequence selected from the group consisting of SEQ ID NO: 50; SEQ ID NO: 51; SEQ ID NO: 52; SEQ ID NO:53; SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56; SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO: 60; SEQ ID NO:61; and SEQ ID NO:62; (b) the reverse complement of said selected sequence; and (c) optionally, a sequence linking sequences (a) and (b); wherein U is substituted for T.

A polynucleotide sequence comprising SEQ ID NO:49.

A method for inhibiting expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising administering to said cell at least one double-stranded RNA effector molecule, preferably 2, 3, 4, 5, 6, or more double-stranded RNA effector molecules, comprising (a) an RNA sequence equivalent to a

hepatitis C virus DNA coding strand sequence selected from the group consisting of sequence position 9510-9531, 9510-9533, 9510-9534, 9510-9535, 9510-9536, 9514-9534, 9514-9535, 9514-9536, 9514-9539, 9514-9540, 9514-9542, 9517-9539, 9517-9540, 9517-9542, 9517-9544, 9518-9539, 9518-9540, 9518-9542, 9518-9544, 9520-9540, 9520-9542, 9520-9544, 9520-9548, 9521-9542, 9521-9544, 9521-9548, 9521-9549, 9522-9542, 9522-9544, 9522-9548, 9522-9549, 9527-9548, 9527-9549, 9527-9551, 9527-9552, 9527-9553, 9527-9555, 9528-9548, 9528-9549, 9528-9551, 9528-9552, 9528-9553, 9528-9555, 9530-9551, 9530-9552, 9530-9553, 9530-9555, 9530-9557, 9530-9558, 9532-9552, 9532-9553, 9532-9555, 9532-9557, 9532-9558, 9532-9559, 9532-9560, 9537-9557, 9537-9558, 9537-9559, 9537-9560, 9537-9561, 9537-9564, 9538-9558, 9538-9559, 9538-9560, 9538-9561, 9538-9564, 9538-9566, 9541-9561, 9541-9564, 9541-9566, 9541-9568, 9541-9569, 9543-9564, 9543-9566, 9543-9568, 9543-9569, 9543-9571, 9545-9566, 9545-9568, 9545-9569, 9545-9571, 9545-9573, 9546-9564, 9546-9566, 9546-9569, 9546-9571, 9546-9573, 9547-9568, 9547-9569, 9547-9571, 9547-9573, 9547-9575, 9550-9571, 9550-9573, 9550-9575, 9550-9577, 9550-9578, 9554-9575, 9554-9577, 9554-9578, 9554-9580, 9556-9577, 9556-9578, 9556-9580, 9556-9584, 9562-9584, 9562-9586, 9562-9587, 9562-9588, 9562-9589, 9563-9584, 9563-9586, 9563-9587, 9563-9588, 9563-9589, 9563-9591, 9565-9586, 9565-9587, 9565-9588, 9565-9589, 9565-9591, 9565-9593, 9567-9587, 9567-9588, 9567-9589, 9567-9591, 9567-9593, 9567-9595, 9570-9591, 9570-9593, 9570-9595, 9570-9596, 9570-9598, 9572-9593, 9572-9595, 9572-9596, 9572-9598, 9574-9595, 9574-9596, 9574-9598, 9574-9601, 9576-9596, 9576-9598, 9576-9601, 9576-9604, 9579-9601, 9579-9604, 9581-9601, 9581-9604, and 9583-9604 and (b) an RNA sequence which is the reverse complement of the selected sequence equivalent to the hepatitis C virus DNA coding strand sequence. In some embodiments, said RNA sequences (a) and (b) are linked by a loop sequence and the double-stranded RNA effector molecule(s) forms a stem-loop or hairpin dsRNA structure. In some aspects, said double-stranded RNA effector molecule(s) are duplex dsRNAs, formed from two separate RNA strands. In some aspects, the method involves administering to a mammalian cell an expression construct encoding one, two, three, four, five or more of said dsRNA effector molecules. In some embodiments designed to target the HCV minus strand, the dsRNA effector molecule will comprise (a) an RNA sequence corresponding to a hepatitis C virus

DNA coding strand sequence as specified above, and (b) the reverse complement of said RNA sequence, optionally linked by a loop sequence. In some embodiments, the dsRNA effector molecule(s) is encoded by an expression construct.

In some aspects the invention relates to a composition for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising at least one double-stranded RNA effector molecule, preferably 2, 3, 4, 5, 6 or more double-stranded RNA effector molecules, or a dsRNA expression construct capable of transcribing one, 2, 3, 4, 5, 6 or more of said dsRNA effector molecules in an in vivo mammalian cell, each of said dsRNA effector molecules comprising (a) an RNA sequence equivalent to a hepatitis C virus DNA coding strand sequence selected from the group consisting of sequence position 9510-9531, 9510-9533, 9510-9534, 9510-9535, 9510-9536, 9514-9534, 9514-9535, 9514-9536, 9514-9539, 9514-9540, 9514-9542, 9517-9539, 9517-9540, 9517-9542, 9517-9544, 9518-9539, 9518-9540, 9518-9542, 9518-9544, 9520-9540, 9520-9542, 9520-9544, 9520-9548, 9521-9542, 9521-9544, 9521-9548, 9521-9549, 9522-9542, 9522-9544, 9522-9548, 9522-9549, 9527-9548, 9527-9549, 9527-9551, 9527-9552, 9527-9553, 9527-9555, 9528-9548, 9528-9549, 9528-9551, 9528-9552, 9528-9553, 9528-9555, 9530-9551, 9530-9552, 9530-9553, 9530-9555, 9530-9557, 9530-9558, 9532-9552, 9532-9553, 9532-9555, 9532-9557, 9532-9558, 9532-9559, 9532-9560, 9537-9557, 9537-9558, 9537-9559, 9537-9560, 9537-9561, 9537-9564, 9538-9558, 9538-9559, 9538-9560, 9538-9561, 9538-9564, 9538-9566, 9541-9561, 9541-9564, 9541-9566, 9541-9568, 9541-9569, 9543-9564, 9543-9566, 9543-9568, 9543-9569, 9543-9571, 9545-9566, 9545-9568, 9545-9569, 9545-9571, 9545-9573, 9546-9564, 9546-9566, 9546-9569, 9546-9571, 9546-9573, 9547-9568, 9547-9569, 9547-9571, 9547-9573, 9547-9575, 9550-9571, 9550-9573, 9550-9575, 9550-9577, 9550-9578, 9554-9575, 9554-9577, 9554-9578, 9554-9580, 9556-9577, 9556-9578, 9556-9580, 9556-9584, 9562-9584, 9562-9586, 9562-9587, 9562-9588, 9562-9589, 9563-9584, 9563-9586, 9563-9587, 9563-9588, 9563-9589, 9563-9591, 9565-9586, 9565-9587, 9565-9588, 9565-9589, 9565-9591, 9565-9593, 9567-9587, 9567-9588, 9567-9589, 9567-9591, 9567-9593, 9567-9595, 9570-9591, 9570-9593, 9570-9595, 9570-9596, 9570-9598, 9572-9593, 9572-9595, 9572-9596, 9572-9598, 9574-9595, 9574-9596, 9574-9598, 9574-9601, 9576-9596, 9576-9598, 9576-9601, 9576-9604, 9579-9601, 9579-9604, 9581-9601, 9581-9604, and 9583-9604 and (b) the reverse complement of said selected

RNA sequence equivalent to the hepatitis C virus DNA coding strand sequence. In some embodiments, said RNA sequences (a) and (b) are linked by a loop sequence, and the double-stranded RNA effector molecule(s) is a single RNA strand which forms a stem-loop or hairpin dsRNA structure. In other embodiments, the dsRNA effector molecule(s) is a duplex dsRNA molecule formed from two separate strands of RNA.

In another aspect, the invention relates to compositions for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising at least one double-stranded RNA effector molecule, preferably 2, 3, 4, 5, 6 or more double-stranded RNA effector molecules, or a dsRNA expression construct capable of expressing one, 2, 3, 4, 5, 6 or more of said dsRNA effector molecules in an in vivo mammalian cell, each of said dsRNA effector molecules comprising (a) a sequence selected from the group consisting of SEQ ID NO: 63; SEQ ID NO: 64; SEQ ID NO: 65; SEQ ID NO:66; SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69; SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO: 73; SEQ ID NO:74; SEQ ID NO:75; and SEQ ID NO:76; (b) the reverse complement of said selected sequence; and (c) optionally, a sequence linking sequences (a) and (b); wherein U is substituted for T. In certain preferred embodiments, the sequence is selected from the group consisting of SEQ ID NO:72; SEQ ID NO: 73; SEQ ID NO:74; SEQ ID NO: 75; and SEQ ID NO:76.

In another aspect, the invention relates to methods for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising administering at least one double-stranded RNA effector molecule, preferably 2, 3, 4, 5, 6 or more double-stranded RNA effector molecules, or a dsRNA expression construct capable of expressing one, 2, 3, 4, 5, 6 or more of said dsRNA effector molecules in an in vivo mammalian cell, each of said dsRNA effector molecules comprising (a) a sequence selected from the group consisting of SEQ ID NO: 63; SEQ ID NO: 64; SEQ ID NO: 65; SEQ ID NO:66; SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69; SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO: 73; SEQ ID NO:74; SEQ ID NO:75; and SEQ ID NO:76; (b) the reverse complement of said selected sequence; and (c) optionally, a sequence linking sequences (a) and (b); wherein U is substituted for T. In certain preferred

embodiments, the sequence is selected from the group consisting of SEQ ID NO:72; SEQ ID NO: 73; SEQ ID NO:74; SEQ ID NO: 75; and SEQ ID NO:76.

In another aspect, the invention relates to a polynucleotide sequence comprising an RNA sequence equivalent to and/or complementary to a hepatitis C virus DNA coding strand sequence selected from the group consisting of sequence position 9510-9531, 9510-9533, 9510-9534, 9510-9535, 9510-9536, 9514-9534, 9514-9535, 9514-9536, 9514-9539, 9514-9540, 9514-9542, 9517-9539, 9517-9540, 9517-9542, 9517-9544, 9518-9539, 9518-9540, 9518-9542, 9518-9544, 9520-9540, 9520-9542, 9520-9544, 9520-9548, 9521-9542, 9521-9544, 9521-9548, 9521-9549, 9522-9542, 9522-9544, 9522-9548, 9522-9549, 9527-9548, 9527-9549, 9527-9551, 9527-9552, 9527-9553, 9527-9555, 9528-9548, 9528-9549, 9528-9551, 9528-9552, 9528-9553, 9528-9555, 9530-9551, 9530-9552, 9530-9553, 9530-9555, 9530-9557, 9530-9558, 9532-9552, 9532-9553, 9532-9555, 9532-9557, 9532-9558, 9532-9559, 9532-9560, 9537-9557, 9537-9558, 9537-9559, 9537-9560, 9537-9561, 9537-9564, 9538-9558, 9538-9559, 9538-9560, 9538-9561, 9538-9564, 9538-9566, 9541-9561, 9541-9564, 9541-9566, 9541-9568, 9541-9569, 9543-9564, 9543-9566, 9543-9568, 9543-9569, 9543-9571, 9545-9566, 9545-9568, 9545-9569, 9545-9571, 9545-9573, 9546-9564, 9546-9566, 9546-9569, 9546-9571, 9546-9573, 9547-9568, 9547-9569, 9547-9571, 9547-9573, 9547-9575, 9550-9571, 9550-9573, 9550-9575, 9550-9577, 9550-9578, 9554-9575, 9554-9577, 9554-9578, 9554-9580, 9556-9577, 9556-9578, 9556-9580, 9556-9584, 9562-9584, 9562-9586, 9562-9587, 9562-9588, 9562-9589, 9563-9584, 9563-9586, 9563-9587, 9563-9588, 9563-9589, 9563-9591, 9565-9586, 9565-9587, 9565-9588, 9565-9589, 9565-9591, 9565-9593, 9567-9587, 9567-9588, 9567-9589, 9567-9591, 9567-9593, 9567-9595, 9570-9591, 9570-9593, 9570-9595, 9570-9596, 9570-9598, 9572-9593, 9572-9595, 9572-9596, 9572-9598, 9574-9595, 9574-9596, 9574-9598, 9574-9601, 9576-9596, 9576-9598, 9576-9601, 9576-9604, 9579-9601, 9579-9604, 9581-9601, 9581-9604, and 9583-9604.

Applicants' invention further provides a method for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising administering to said cell a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and

SEQ ID NO:10; wherein U is substituted for T. In a preferred embodiment of the method, effector sequences from more than one SEQ ID sequence may be administered to the same cell, and/or more than one effector sequence from within the same SEQ ID sequence may be administered to the same cell.

Applicants further provide a method for inhibiting expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising administering to said cell a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:27; wherein U is substituted for T. In a preferred embodiment of this aspect of the method, effector molecules from both SEQ ID NO:11 and SEQ ID NO:12 may be administered to the same cell; or from both SEQ ID NO: 11 and SEQ ID NO:27; or from both SEQ ID NO: 12 and SEQ ID NO:27; or from each of SEQ ID NO: 11, SEQ ID NO:12, and SEQ ID NO:27, are administered to the same cell; and/or more than one effector molecule from within the same SEQ ID NO may be administered to the same cell.

Applicants further provide a method for inhibiting expression of both a polynucleotide sequence of hepatitis B virus and a polynucleotide sequence of hepatitis C virus in the same *in vivo* mammalian cell, comprising administering to said cell a double-stranded RNA effector molecule comprising a first at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; wherein U is substituted for T; and a double-stranded RNA effector molecule comprising a second at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:27; wherein U is substituted for T. In preferred embodiments of this aspect of the invention, effector molecules from more than one of SEQ ID NO:1 through SEQ ID NO:10 may be administered to the same cell; and/or effector molecules from both SEQ ID NO:11 and SEQ ID NO:12; or from both SEQ ID NO: 11 and SEQ ID NO:27; or from both SEQ ID NO: 12 and SEQ ID NO:27; or from SEQ ID NO: 11, SEQ ID NO:12 and SEQ ID NO:27; may be

administered to the same cell; and/or more than one effector molecules from within the same SEQ ID NO may be administered to the same cell.

Applicants further provide a composition for inhibiting the expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; wherein U is substituted for T. Preferred embodiments of the composition include wherein effector molecules from more than one of SEQ ID NO:1 through SEQ ID NO:10 are present in the composition; and/or wherein more than one effector molecule from within the same SEQ ID NO is present in the composition.

Applicants further provide a composition for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:12 and SEQ ID NO:27; wherein U is substituted for T. Preferred embodiments of the composition include wherein effector molecules from both SEQ ID NO:11 and SEQ ID NO:12 are present in the composition; or from both SEQ ID NO: 11 and SEQ ID NO:27; or from both SEQ ID NO: 12 and SEQ ID NO:27; or from each of SEQ ID NO: 11, SEQ ID NO:12, and SEQ ID NO:27, are present in the same composition, and/or wherein more than one effector molecule from within the same SEQ ID NO may be present in the composition.

Applicants further provide a composition for inhibiting the expression of both a polynucleotide sequence of hepatitis B virus and a polynucleotide sequence of hepatitis C virus in a single *in vivo* mammalian cell comprising a double-stranded RNA effector molecule comprising a first at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; wherein U is substituted for T; and a double-stranded RNA effector molecule comprising a second at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:12 and SEQ ID

NO:27; wherein U is substituted for T. Preferred embodiments of the composition include wherein effector molecules from more than one of SEQ ID NO:1 through SEQ ID NO:10 are present in the composition; and/or wherein effector molecules from both SEQ ID NO:11 and SEQ ID NO:12; or from both SEQ ID NO: 11 and SEQ ID NO:27; or from both SEQ ID NO: 12 and SEQ ID NO:27; or from each of SEQ ID NO: 11, SEQ ID NO:12, and SEQ ID NO:27, are present in the composition; and/or wherein more than one effector sequence from within the same SEQ ID NO may be present in the composition.

In particularly preferred embodiments of the above methods and compositions of the invention, the polynucleotide sequence is present within a double-stranded region of an RNA, and the mammalian cell is a human cell.

Further provided are compositions for inhibiting the expression of a polynucleotide sequence of hepatitis B virus and/or a polynucleotide sequence of hepatitis C virus in mammalian cells, wherein said compositions comprise an at least 19 contiguous nucleotide sequence selected from within SEQ ID NO:1 through SEQ ID NO:12, and SEQ ID NO:27; the complement sequences of said SEQ ID NO:1 through SEQ ID NO:12, and SEQ ID NO: 27 sequences, and mixtures of these sequences. In this embodiment of the invention, the "an at least 19 contiguous nucleotide sequence" is preferably DNA, and the mammalian cell is preferably human. Also provided are expression constructs comprising any of the aforementioned compositions and a mammalian cell comprising said expression constructs.

Replace the paragraph beginning at page 6, line 27, with the following amended paragraph:

Another aspect provides for a polynucleotide sequence comprising a sequence selected from SEQ ID NO:14 through SEQ ID NO:26. Another aspect of the invention provides for polynucleotide sequence comprising nucleotides 1-19, 1-20, 1-21, 2-20, 2-21, or 3-21 of a sequence selected from SEQ ID NO:14 through SEQ ID NO:26. Another aspect of the invention provides for a polynucleotide sequence comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence selected from SEQ ID NO:27 through SEQ ID NO:44.

Replace the paragraph beginning at page 7, line 4, with the following amended paragraph:

Another aspect provides a composition for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in a mammalian cell, comprising a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within SEQ ID NO:27; wherein U is substituted for T.

Add, at page 7, line 9, the following new paragraph:

In various aspects of the foregoing methods and compositions, the *in vivo* mammalian cell is an *in vivo* human cell.

Replace the paragraph beginning at page 8, line 6, with the following amended paragraph:

SEQ ID NO:48 represents a 5' segment of the hepatitis C virus sequence (corresponds to positions 36 to 358 in Genbank Accession Number AJ238799, with 2 base changes, C to G at AJ238799 position 204 and G to A at AJ238799 position 357).

Add, at page 8, line 8, the following new section:

SEQ ID NO:49 represents an eiRNA (shRNA) molecule to a conserved HBV sequence.
SEQ ID NO:50 through SEQ ID NO:62 represent the first 21 nucleotides of SEQ ID NOs: 14-23, 25-26, and 49.
SEQ ID NO:63 through SEQ ID NO:71 represent the first 21 nucleotides of SEQ ID NOs: 28-36.
SEQ ID NO:72 through SEQ ID NO:76 represent highly conserved coding region sequitopes from the 5' and 3' untranslated regions of HCV.

SEQ ID NO:77 through SEQ ID NO:109 represent highly conserved HCV sequences from the 5' UTR of the HCV (SEQ ID NO: 11).

Add, at page 9, line 23, the following new paragraph:

Figure 15 is a table of additional conserved HCV genome sequence segments suitable for generating dsRNA effector molecules which inhibit the expression of polynucleotide sequences of hepatitis C virus, including expressed shRNA for gene silencing. Each sequence represents a DNA coding strand sequence in standard 5' to 3' polarity which (together with its reverse complement) can be utilized to transcribe or design a double-stranded RNA effector molecule, e.g., an shRNA or duplex dsRNA molecule targeted to degrade the negative strand of HCV RNA. E.g.,an DNA sequence, followed by a loop sequence (e.g., a 9 base loop sequence as described elsewhere herein), followed by the reverse complement of the sequence given in the table, may be incorporated into an expression construct under the control of an appropriate promoter. The shRNA molecule transcribed from such an expression construct is expected to inhibit expression of HCV polynucleotide sequences and/or mediate dsRNA silencing of HCV. For example, in the case of the 22 base sequence shown for positions 9545-9566, a construct is made to contain a 53 bp insert, comprising the 22 base sequence of 9545-9566, a linker or loop sequence, and the reverse complement of the 9545-9566 sequence, preferably under the control of an RNA polymerase III promoter and ending with an RNA polymerase III terminator, e.g., a run of 4, 5, or more T residues. The RNA equivalent of this sequence, having U's instead of T's, would read (in the 5' to 3' direction):

AAAGGUCCGUGAGCCGCUUGAC-XXXXXXXXX-GUCAAGCGGUCACGGACCUU
U

where X represents bases of the loop that are unable to form stable base pairs with any other portion of the 53 bp shRNA sequence. The loop may vary considerably, however, as to both length and nucleotide sequence, so long as the formation of the double-stranded "stem" region of the hairpin is not adversely affected. Thus, in

expression constructs that are the subject of this invention, the sequence element above beginning at the end which reads 5' AAAGGT is cloned into an appropriate vector downstream from and operably linked to the promoter. As described elsewhere herein, in preferred embodiments, two, three, four, five, six, seven, or more of the shRNAs encoded by these sequences, optionally, together with other anti-HCV, and/or HBV sequences described herein, are coded into and expressed by a single dsRNA expression vector. In one aspect, each of said multiple stem-loop or shRNA molecules is encoded in a single expression vector within a different expression cassette, each operably linked to a promoter and a terminator, preferably a polymerase III promoter, which may be the same or different. In another aspect, two or more hairpin dsRNA molecules may be expressed from a single promoter, as e.g., a bi-fingered molecule in which a single transcribed RNA strand comprises two such shRNA sequences separated by an unrelated linker sequence. Such constructs, in which a single expression vector provides a mammalian cell with two, three, four, five or more independent dsRNA effector molecules against an HCV and/or HBV target polynucleotide, are particularly desirable for pharmaceutical applications. An alternative means of dsRNA-mediated silencing may be accomplished by preparing shRNAs or duplex dsRNAs corresponding to the identified sequences by chemical synthesis or *in vitro* expression and delivering them into a cell in order to achieve inhibition of HCV and/or HBV polynucleotide sequences.

Replace the paragraph beginning at page 10, line 30, with the following amended paragraph:

In GenBank version 132.0 there are more then 4500 HBV sequences and 340 HBV complete genome sequences (317 Human isolates, 22 isolates from other primates and one woodchuck HBV isolate). This variability constitutes a serious challenge for sequence-specific pharmaceutical approaches such as RNAi. In order to identify conserved sequences suitable for RNAi applications, a comparison between all the complete genomes was carried out using a modified version of ClustalW. Two multiple alignment schemes were generated: the first included all 339 HBV complete genome sequences and the second was limited to all Human

HBV isolates. The multiple alignment results were parsed and a table that included scores for sequence conservation at each position in the HBV genome was generated. A sliding window search to identify the longest region of sequence conservation larger then 19 nt in length was created. Three major conserved regions were identified and mapped to GenBank accession no.: AF090840, a Human HBV isolate. The conserved HBV sequences were screened against Genbank sequences of both human genomic and cDNA libraries (Human chromosomes database). It was found that 21 nucleotide and longer segments selected as a permuted "window" from within the conserved regions were unique to HBV, i.e. no perfect sequence matches exist between any 21 nt or longer HBV conserved segments and the available sequence databases of human chromosomal and RNA sequences. For human therapeutic purposes, assuring that homologous human sequences are not inadvertently silenced is as important as selecting conserved viral sequences for RNAi.

Replace the paragraph beginning at page 12, line 13, with the following amended paragraph:

In GenBank version 134.0 there are more then 20,000 HCV sequences and 93 HCV complete genome sequences. A comparison between all the complete genomes was carried out using a modified version of ClustalW. The multiple alignment result was parsed and a table that included scores for sequence conservation at each position in the HCV genome was generated. A sliding window search to identify the longest region of sequence conservation larger then 19 nt in length was created. Three major conserved regions were identified and mapped to GenBank RefSeq (reference sequence) accession no.: NC_004102 this is GenBank annotated HCV complete genome. The three major conserved regions include a portion of the 3' untranslated region of the virus, already described in the literature to be well-conserved among viral isolates. See, e.g., US Patent 5,874,565, "Nucleic Acids Comprising a Highly Conserved Novel 3' Terminal Sequence Element of the Hepatitis C Virus." However, the instant disclosure represents a comprehensive and detailed analysis of these conserved regions to the extent that permitted the discovery and evaluation of multiple short segments suitable for use alone and in

combination as a therapeutic for silencing HCV among a diverse patient population. The conserved sequences were screened against Genbank sequences of both human genomic and cDNA libraries (human chromosomes database), and the series of permuted HCV segments greater than 20 bases long with no homology to the human sequence databases were identified.

Replace the paragraph beginning at page 13, line 12, with the following amended paragraph:

Since the length of a contiguous dsRNA sequence capable of association with and activation of RISC (RNA-induced silencing complex), is generally considered to be 19-27 base pairs, the identified conserved HBV and HCV sequences were compared with both human genomic libraries and, perhaps even more importantly, with human cDNA libraries as described above. Since human cDNA libraries represent expressed sequences that appear in mRNAs, such mRNA sequences would be especially vulnerable to silencing by homologous dsRNA sequences provided to a cell.

Replace the paragraph beginning at page 15, line 4, with the following amended paragraph:

HBV_Conserved_Region_5

[C(69%)/del(31%)][G(69%)/del(31%)]A[G(85%)/T(11%)/C(4%)]GCAGG TCCCCTAGAAGAAGAACTCCCTCGCCTCGCAGACG[C(61%)/A(39%)]AG[A(62%)/G(38%)]TCTCAATCG[C(88%)/A(12%)]CGCGTCGCAGAAGATCTCAAT[C(92%)/T(8%)]TCGGGAATCT[C(88%)/T(12%)]AATGTTAGTAT

Replace the section beginning at page 17, line 19, and ending at page 18, line 5, with the following amended section:

SEQ ID NO:5 HBV

CGA**b**GCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCGCAGACG **mA**G**r**TCTCAATCG**m**CGCGTCGCAGAAGATCTCAAT**y**TCGGGAATCT**y**AATGTTA GTAT

SEQ ID NO:6 HBV

A**b**GCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCGCAGACG**mA** G**r**TCTCAATCG**m**CGCGTCGCAGAAGATCTCAAT**y**TCGGGAATCT**y**AATGTTAGTA T

SEQ ID NO:7 HBV

CA**b**GCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCGCAGACG**m** **A**G**r**TCTCAATCG**m**CGCGTCGCAGAAGATCTCAAT**y**TCGGGAATCT**y**AATGTTAGT AT

SEQ ID NO:8 HBV

GAbGCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCGCAGACG**m** AG**r**TCTCAATCG**m**CGCGTCGCAGAAGATCTCAAT**y**TCGGGAATCT**y**AATGTTAGT AT

Replace the section beginning at page 19, line 25, and ending at page 24, line 21, with the following amended section:

By "dsRNA" or "dsRNA effector molecule" is meant a nucleic acid containing a region of two or more nucleotides that are in a double stranded conformation. It is envisioned that the conserved viral sequences of the invention may be utilized in any of the many compositions of "dsRNA effector molecules" known in the art or subsequently developed which act through a dsRNA-mediated gene silencing or RNAi mechanism, including, e.g., "hairpin" or stem-loop double-stranded RNA effector molecules in which a single RNA strand with self-complementary sequences is capable of assuming a double-stranded conformation, or duplex dsRNA effector molecules comprising two separate strands of RNA,. In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides

and deoxynucleotides, such as the RNA/DNA hybrids disclosed, for example, by WO 00/63364, filed April 19, 2000, or U.S.S.N. 60/130,377, filed April 21, 1999. The dsRNA or dsRNA effector molecule may be a single molecule with a region of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In various embodiments, a dsRNA that consists of a single molecule consists entirely of ribonucleotides or includes a region of ribonucleotides that is complementary to a region of deoxyribonucleotides. Alternatively, the dsRNA may include two different strands that have a region of complementarity to each other. In various embodiments, both strands consist entirely of ribonucleotides, one strand consists entirely of ribonucleotides and one strand consists entirely of deoxyribonucleotides, or one or both strands contain a mixture of ribonucleotides and deoxyribonucleotides. Desirably, the regions of complementarity are at least 70, 80, 90, 95, 98, or 100% complementary to each other and to a target nucleic acid sequence. Desirably, the region of the dsRNA that is present in a double stranded conformation includes at least 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 75,100, 200, 500, 1000, 2000 or 5000 nucleotides or includes all of the nucleotides in a cDNA or other target nucleic acid sequence being represented in the dsRNA. In some embodiments, the dsRNA does not contain any single stranded regions, such as single stranded ends, or the dsRNA is a hairpin. In other embodiments, the dsRNA has one or more single stranded regions or overhangs. Desirable RNA/DNA hybrids include a DNA strand or region that is an antisense strand or region (*e.g*, has at least 70, 80, 90, 95, 98, or 100% complementarity to a target nucleic acid) and an RNA strand or region that is a sense strand or region (*e.g*, has at least 70, 80, 90, 95, 98, or 100% identity to a target nucleic acid), and vice versa. In various embodiments, the RNA/DNA hybrid is made *in vitro* using enzymatic or chemical synthetic methods such as those described herein or those described in WO 00/63364, filed April 19, 2000, or U.S.S.N. 60/130,377, filed April 21, 1999. In other embodiments, a DNA strand synthesized *in vitro* is complexed with an RNA strand made *in vivo* or *in vitro* before, after, or concurrent with the transformation of the DNA strand into the cell. In yet other embodiments, the dsRNA is a single circular nucleic acid containing a sense and an antisense region, or the dsRNA includes a circular nucleic acid and either a second circular nucleic acid or a linear nucleic acid (see, for example, WO 00/63364,

filed April 19, 2000, or U.S.S.N. 60/130,377, filed April 21, 1999.) Exemplary circular nucleic acids include lariat structures in which the free 5' phosphoryl group of a nucleotide becomes linked to the 2' hydroxyl group of another nucleotide in a loop back fashion.

In other embodiments, the dsRNA includes one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group) or contains an alkoxy group (such as a methoxy group) which increases the half-life of the dsRNA *in vitro* or *in vivo* compared to the corresponding dsRNA in which the corresponding 2' position contains a hydrogen or an hydroxyl group. In yet other embodiments, the dsRNA includes one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The dsRNAs may also be chemically modified nucleic acid molecules as taught in U.S. Patent No. 6,673,661. In other embodiments, the dsRNA contains one or two capped strands, as disclosed, for example, by WO 00/63364, filed April 19, 2000, or U.S.S.N. 60/130,377, filed April 21, 1999. In other embodiments, the dsRNA contains coding sequence or non-coding sequence, for example, a regulatory sequence (e.g., a transcription factor binding site, a promoter, or a 5' or 3' untranslated region (UTR) of an mRNA). Additionally, the dsRNA can be any of the at least partially dsRNA molecules disclosed in WO 00/63364, filed April 19, 2000 (see, for example, pages 8-22), as well as any of the dsRNA molecules described in US Provisional Application 60/399,998 filed July 31, 2002, and PCT/US2003/024028, filed 31-Jul-2003; and US Provisional Application 60/419,532 filed October 18, 2002, and PCT/US2003/033466, filed 20-Oct-2003, the teaching of which is hereby incorporated by reference. Any of the dsRNAs may be expressed *in vitro* or *in vivo* using the methods described herein or standard methods, such as those described in WO 00/63364, filed April 19, 2000 (see, for example, pages 16-22). In some preferred embodiments, multiple anti-HBV and/or anti-HCV dsRNA effector molecules of the invention are transcribed in a mammalian cell from one or more expression constructs each comprising multiple polymerase III promoter expression cassettes as described in more detail in US 60/603622; US 60/629942; and PCT/US05/29976 filed 23-August-2005; "Multiple Polymerase III Promoter Expression Constructs"; the teaching of which is incorporated by reference.

dsRNA "Hairpin" Constructs or dsRNA "Hairpin" Expression Vectors: Constructs encoding a unimolecular hairpin dsRNA are more desirable for some applications than constructs encoding duplex dsRNA (i.e., dsRNA composed of one RNA molecule with a sense region and a separate RNA molecule with an antisense region) because the single-stranded RNA with inverted repeat sequences more efficiently forms a dsRNA hairpin structure. This greater efficiency is due in part to the occurrence of transcriptional interference arising in vectors containing converging promoters that generate duplex dsRNA. Transcriptional interference results in the incomplete synthesis of each RNA strand thereby reducing the number of complete sense and antisense strands that can base-pair with each other and form duplexes. Transcriptional interference can be overcome, if desired, through the use of (i) a two vector system in which one vector encodes the sense RNA and the second vector encodes the antisense RNA, (ii) a bicistronic vector in which the individual strands are encoded by the same plasmid but through the use of separate cistrons, or (iii) a single promoter vector that encodes a hairpin dsRNA, i.e., an RNA in which the sense and antisense sequences are encoded within the same RNA molecule. Hairpin-expressing vectors have some advantages relative to the duplex vectors. For example, in vectors that encode a duplex RNA, the RNA strands need to find and base-pair with their complementary counterparts soon after transcription. If this hybridization does not happen, the individual RNA strands diffuse away from the transcription template and the local concentration of sense strands with respect to antisense strands is decreased. This effect is greater for RNA that is transcribed intracellularly compared to RNA transcribed *in vitro* due to the lower levels of template per cell. Moreover, RNA folds by nearest neighbor rules, resulting in RNA molecules that are folded co-transcriptionally (i.e., folded as they are transcribed). Some percentage of completed RNA transcripts is therefore unavailable for base-pairing with a complementary second RNA because of intra-molecular base-pairing in these molecules. The percentage of such unavailable molecules increases with time following their transcription. These molecules may never form a duplex because they are already in a stably folded structure. In a hairpin RNA, an RNA sequence is always in close physical proximity to its complementary RNA. Since RNA structure is not static, as the RNA transiently unfolds, its complementary sequence is immediately available and can participate in base-pairing because it is

so close. Once formed, the hairpin structure is predicted to be more stable than the original non-hairpin structure. Especially desirable are, e.g., "forced" hairpin constructs, partial hairpins capable of being extended by RNA-dependent RNA polymerase to form dsRNA hairpins, as taught in USSN 60/399,998P, filed 31-Jul-2002; and PCT/US2003/024028, "Double Stranded RNA Structures and Constructs and Methods for Generating and Using the Same," filed 31-Jul-2003; as well as the "udderly" structured hairpins, hairpins with mismatched regions, and multiepitope constructs as taught in USSN 60/419,532, filed 18-Oct-2002, and PCT/US2003/033466, "Double-Stranded RNA Structures and Constructs, and Methods for Generating and Using the Same," filed 20-Oct-2003.

By "short dsRNA" is meant a dsRNA that has about 200, 100, 75, 50, 45, 40, 35, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20 or 19 contiguous nucleotides in length that are in a double stranded conformation. Desirably, the short dsRNA comprises a double-stranded region of at least 19 contiguous basepairs in length identical/complementary to a target sequence to be inhbiited. In desirable embodiments, the double stranded region is between 19 to 50, 19 to 40, 19 to 30, 19 to 25, 20 to 25, 21 to 23, 25 to 30, or 30 to 40 contiguous basepairs in length, inclusive. In some embodiments, the short dsRNA is between 30 to 50, 50 to 100, 100 to 200, 200 to 300, 400 to 500, 500 to 700, 700 to 1000, 1000 to 2000, or 2000 to 5000 nucleotides in length, inclusive and has a double stranded region that is between 38 and 60 contiguous basepairs in length, inclusive. In one embodiment, the short dsRNA is completely double stranded. In some embodiments, the short dsRNA is between 11 and 30 nucleotides in length, and the entire dsRNA is double stranded. In other embodiments, the short dsRNA has one or two single stranded regions. In some embodiments, the short dsRNA is a "shRNA" or "short-hairpin RNA" or "shRNA effector molecule" or "dsRNA hairpin", meaning an RNA molecule of less than approximately 400 to 500 nucleotides (nt) in length, preferably less than 100 to 200 nt in length, in which at least one stretch of at least about 15 to 100 nucleotides (preferably 17 to 50 nt; more preferably 19 to 29 nt) is base paired with a complementary sequence located on the same RNA molecule, and where said sequence and complementary sequence are separated by an unpaired region of at least about 4 to 7 nucleotides (preferably about 9 to about 15 nucleotides) which forms a single-stranded loop above the stem structure created by the two regions of

base complementarity. The shRNA molecules comprise at least one stem-loop structure comprising a double-stranded stem region of about 17 to about 100 bp; about 17 to about 50 bp; about 40 to about 100 bp; about 18 to about 40 bp; or from about 19 to about 29 bp; homologous and complementary to a target sequence to be inhibited; and an unpaired loop region of at least about 4 to 7 nucleotides; preferably about 9 to about 15 nucleotides, which forms a single-stranded loop above the stem structure created by the two regions of base complementarity. Included shRNAs are dual or bi-finger (i.e., having two stem-loop structures) and multi-finger hairpin dsRNAs (having multiple stem-loop structures), in which the RNA molecule comprises two or more of such stem-loop structures separated by single-stranded spacer regions. In some embodiments, an expression construct may be used to express one or more of such shRNA molecules in a mammalian cell, including multiple copies of the same, and/or one or more, including multiple different, short hairpin RNA molecules. Short hairpin RNA molecules considered to be the "same" as each other are those that comprise only the same double-stranded sequence, and short hairpin RNA molecules considered to be "different" from each other will comprise different double-stranded sequences, regardless of whether the sequences to be targeted by each different double-stranded sequence are within the same, or a different gene, such as, e.g., sequences of a promoter region and of a transcribed region (mRNA) of the same gene, or sequences of two different genes.

In particular embodiments, the short dsRNA binds PKR or another protein in a dsRNA-mediated stress response pathway. Desirably, such a short dsRNA inhibits the dimerization and activation of PKR by at least 20, 40, 60, 80, 90, or 100%. In some desirable embodiments, the short dsRNA inhibits the binding of a long dsRNA to PKR or another component of a dsRNA-mediated stress response pathway by at least 20, 40, 60, 80, 90, or 100%. See also the teaching of USSN 10/425,006, filed 28-Apr-2003, "Methods of Silencing Genes Without Inducing Toxicity", Pachuk, as to utilization of short dsRNAs in conjunction with other dsRNAs to avoid dsRNA-mediated toxicity. The applicants have demonstrated, however, that dsRNA molecules, even long dsRNA molecules, are in general unlikely to evoke a significant dsRNA stress response, including a PKR or interferon or "panic" response, if they are expressed intracellularly in the mammalian (or other vertebrate) cell in which the RNAi effect is desired. See, e.g., US 2002/0132257, "Use of post-transcriptional

gene silencing for identifying nucleic acid sequences that modulate the function of a cell". Accordingly, such "expressed interfering RNA molecules" or "eiRNA" molecules and "eiRNA expression constructs", i.e., dsRNA molecules (or the corresponding dsRNA expression constructs) expressed intracellularly or endogenously in vivo within the mammalian cell in which dsRNA gene silencing or RNAi is induced, are preferred in some aspects of the invention.

By "at least 19 contiguous base pair nucleotide sequence" is meant that a nucleotide sequence can start at any nucleotide within one of the disclosed sequences, so long as the start site is capable of producing a polynucleotide of at least 19 contiguous base pairs. For example, an at least 19 contiguous base pair nucleotide sequence can comprise nucleotide 1 through nucleotide 19, nucleotide 2 through nucleotide 20, nucleotide 3 through nucleotide 21, and so forth to produce a 19mer. Thus, a 20mer can comprise nucleotide 1 through nucleotide 20, nucleotide 2 through nucleotide 21, nucleotide 3 through nucleotide 22, and so forth. Similar sequences above 20 contiguous nucleotides, e,g., 21, 22, 23, 24, 25, 26, 27, etc. selected from within the conserved sequences are envisioned. Such a sequence of at least 19 contiguous nucleotides (in double-stranded conformation with its complement) is "an at least 19 contiguous base pair sequence" and may be present as a duplex dsRNA, within a dsRNA hairpin, or encoded in a dsRNA expression construct.

Replace the section beginning at page 25, line 3, and ending at page 26, line 16, with the following amended section:

By an "expression construct" is meant any double-stranded DNA or double-stranded RNA designed to transcribe an RNA, e.g., a construct that contains at least one promoter operably linked to a downstream gene or coding region of interest (*e.g.*, a cDNA or genomic DNA fragment that encodes a protein, or any RNA of interest). Transfection or transformation of the expression construct into a recipient cell allows the cell to express RNA or protein encoded by the expression construct. An expression construct may be a genetically engineered plasmid, virus, or artificial chromosome derived from, for example, a bacteriophage, adenovirus, retrovirus, poxvirus, or herpesvirus. An expression construct does not have to be replicable in a

living cell, but may be made synthetically. An expression construct or expression vector engineered to express a double-stranded RNA effector molecule or dsRNA molecule is a "dsRNA expression construct" or "dsRNA expression vector".

In one embodiment of the invention, a recombinant expression vector or expression construct is engineered to express multiple, e.g., three, four, five or more short hairpin dsRNA effector molecules, each expressed from a different expression cassette comprising a polymerase III promoter, one or more, including all of which, may be different from the others. In one aspect of the invention, a recombinant expression vector transcribing three, four, five or more different shRNA molecules (each comprising a double-stranded "stem" region comprising at least 19 contiguous basepairs from/complementary to a conserved HBV and/or HCV sequence) is used to inhibit replication of hepatitis B virus (HBV) and/or hepatitis C virus (HCV). In one embodiment, each shRNA molecule is expressed under the control of a polymerase III promoter, e.g., 7SK, H1, and U6, which may be the same of different. Such dsRNA expression constructs comprising multiple polymerase III expression cassettes are described in greater detail in PCT/US05/29976, "Multiple Polymerase III Promoter Expression Constructs", the teaching of which is hereby incorporated by reference. In one aspect, a recombinant expression vector or expression construct of the invention may express one or more bi-fingered or multi-fingered dsRNA hairpin molecules from one or more polymerase III promoter-driven transcription units as well as one or more single hairpin dsRNA molecules from one or more polymerase III promoter-driven transcription units. It will be understood that in any of said expression constructs transcribing a hairpin dsRNA from a polymerase III promoter, the hairpin dsRNA may be a single hairpin dsRNA or a bi-fingered, or multi-fingered dsRNA hairpin as described in WO2004/035765, published 29-Apr-2004, or a partial or forced hairpin structure as described in WO2004/011624, published 5-Feb-2004, the teaching of which is incorporated herein by reference.

By "operably linked" is meant that a nucleic acid sequence or molecule and one or more regulatory sequences (e.g., a promoter, enhancer, repressor, terminator) are connected in such a way as to permit transcription of an RNA molecule, e.g., a single-stranded RNA molecule such as a sense, antisense, a dsRNA hairpin, or an mRNA, or permit expression and translation and/or secretion of

the product (i.e., a polypeptide) of the nucleic acid molecule when the appropriate molecules are bound to the regulatory sequences.

By a "promoter" is meant a nucleic acid sequence sufficient to direct transcription of a covalently linked nucleic acid molecule. Also included in this definition are those transcription control elements (*e.g.*, enhancers) that are sufficient to render promoter-dependent gene expression controllable in a cell type-specific, tissue-specific, or temporal-specific manner, or that are inducible by external signals or agents; such elements, which are well-known to skilled artisans, may be found in a 5' or 3' region of a gene or within an intron. See, e.g., published U.S. Patent Application No. 2005/0130184 A1, 16-June-2005, Xu et al., directed to modified polymerase III promoters which utilize polymerase II enhancer elements, as well as Published U.S. Patent Application No. 2005/0130919 A1, 16-June-2005, Xu et al., directed to regulatable polymerase III and polymerase II promoters, the teaching of which is hereby incorporated by reference. Desirably a promoter is operably linked to a nucleic acid sequence, for example, a cDNA or a gene sequence, or a sequence encoding a dsRNA, e.g., a shRNA, in such a way as to permit expression of the nucleic acid sequence.

The RNA molecule according to this invention may be delivered to the mammalian cell or extracellular pathogen present in the mammalian cell in the composition as a dsRNA effector molecule or partially double stranded RNA sequence, or RNA/DNA hybrid, which was made *in vitro* by conventional enzymatic synthetic methods using, for example, the bacteriophage T7, T3 or SP6 RNA polymerases according to the conventional methods described by such texts as the Promega Protocols and Applications Guide, (3rd ed. 1996), eds. Doyle, ISBN No. 1 57 Alternatively these molecules may be made by chemical synthetic methods *in vitro* [see, e.g., Q. Xu et al., Nucleic Acids Res., 24(18):3643-4 (Sept. 1996); N. Naryshkin et al., Bioorg. Khim., 22(9):691-8 (Sept. 1996); J. A. Grasby et al., Nucleic Acids Res., 21(19):4444-50 (Sept 1993); C. Chaix et al., Nucleic Acids Res. 17:7381-93 (1989); S.H. Chou et al., Biochem., 28(6):2422-35 (Mar. 1989); 0. Odal el al., Nucleic Acids Symp. Ser., 21:105-6 (1989); N.A. Naryshkin et al., Bioorg. Khim, 22(9):691-8 (Sept. 1996); S. Sun et al., RNA, 3(11):1352-1363 (Nov. 1997); X. Zhang et al., Nucleic Acids Res., 25(20):3980-3 (Oct. 1997); S. M. Grvaznov el al., Nucleic Acids Res., 2-6 (18):4160-7 (Sept. 1998); M. Kadokura et al., Nucleic Acids

Symp. Ser., 37:77-8 (1997); A. Davison et al., Biorned. Pept. Proteins. Nucleic Acids, 2(I):1-6 (1996); and A. V. Mudrakovskaia et al., Bioorg. Khirn., 17(6):819-22 (Jun. 1991)].

Replace the paragraph beginning at page 27, line 6, with the following amended paragraph:

Preferably the single stranded RNA sense or anti-sense strand forms a hairpin at one or both termini. Desirably, the single stranded RNA sense or anti-sense strand forms a hairpin at some intermediate portion between the termini. Such a single stranded RNA sense or anti-sense strand may also be designed to fold back upon itself to become partially double stranded *in vitro* or *in vivo*. Yet another embodiment of an extant RNA molecule as the effective agent used in the compositions is a single stranded RNA sequence comprising both a sense polynucleotide sequence and an antisense polynucleotide sequence, optionally separated by a non-base paired polynucleotide sequence. Preferably, this single stranded RNA sequence has the ability to become double-stranded once it is in the cell, or *in vitro* during its synthesis. In desirable embodiments, a sequence of at least about 19 to 29 contiguous basepairs will assume a double-stranded conformation. In desirable embodiments, the double-stranded region will include an at least about 19 contiguous basepair sequence identical/complementary to a target nucleotide sequence to be downregulated or inhibited.

Replace the section beginning at page 28, line 3, and ending at page 29, line 27, with the following amended section:

Alternatively, the RNA molecule may be formed *in vivo* and thus delivered by a "delivery agent" which generates such a partially double-stranded RNA molecule *in vivo* after delivery of the agent to the mammalian cell or to the mammal. Thus, the agent which forms the composition of this invention is, in one embodiment, a double stranded DNA molecule "encoding" one of the above-described RNA molecules, e.g., a dsRNA expression vector or expression construct. The DNA agent provides the nucleotide sequence which is transcribed within the cell to become a double

stranded RNA. In another embodiment, the DNA sequence provides a deoxyribonucleotide sequence which within the cell is transcribed into the above-described single stranded RNA sense or anti-sense strand, which optionally forms a hairpin at one or both termini or folds back upon itself to become partially double stranded. The DNA molecule which is the delivery agent of the composition can provide a single stranded RNA sequence comprising both a sense polynucleotide sequence and an anti-sense poiynucleotide sequence, optionally separated by a nonbase paired polynucleotide sequence, and wherein the single stranded RNA sequence has the ability to become double-stranded. Alternatively, the DNA molecule which is the delivery agent provides for the transcription of the above-described circular RNA molecule that optionally forms a rod structure or partial double strand *in vivo*. The DNA molecule may also provide for the *in vivo* production of an RNA/DNA hybrid as described above, or a duplex containing one RNA strand and one DNA strand. These various DNA molecules may be designed by resort to conventional techniques such as those described in Sambrook, cited above or in the Promega reference, cited above.

A latter delivery agent of the present invention, which enables the formation in the mammalian cell of any of the above-described RNA molecules, can be a DNA single stranded or double stranded plasmid or vector. Expression vectors designed to produce RNAs as described herein *in vitro* or *in vivo* may contain sequences under the control of any RNA polymerase, including mitochondrial RNA polymerase, RNA pol I, RNA pol II, and RNA pol III, and viral polymerases, and bacteriophage polymerases such as T7 and Sp6. Desirably, expression vectors designed for in vivo expression of dsRNA effector molecules within a mammalian cell may be designed to utilize an endogenous mammalian polymerase such as an RNA polymerase I, RNA polymerase II, RNA polymerase III, and/or a mitochondrial polymerase. Expression vectors utilizing cognate promoter(s), e.g., polymerase III promoters such as U6, H1, or 7SK, in order to effect transcription by RNA polymerase III can readily be designed. Preferred for expression of short RNA molecules less than about 400 to 500 nucleotides in length are RNA polymerase III promoters. In some aspects, an "RNA polymerase III promoter" or "RNA pol III promoter" or "polymerase III promoter" or "pol III promoter" is preferred, meaning any invertebrate, vertebrate, or mammalian promoter, e.g., human, murine, porcine, bovine, primate, simian, etc.

that, in its native context in a cell, associates or interacts with RNA polymerase III to transcribe its operably linked gene, or any variant thereof, natural or engineered, that will interact in a selected host cell with an RNA polymerase III to transcribe an operably linked nucleic acid sequence. Preferred in some applications are the Type III RNA pol III promoters including U6, H1, 7SK, and MRP which exist in the 5' flanking region, include TATA boxes, and lack internal promoter sequences. One reason RNA Pol III promoters are especially desirable for expression of small engineered RNA transcripts is that RNA Pol III termination, unlike RNA polymerase II termination, occurs efficiently and precisely at a short run of thymine residues in the DNA coding strand, without other protein factors, T_4 and T_5 being the shortest Pol III termination signals in yeast and mammals, with oligo (dT) terminators longer than T_5 being very rare in mammals. Accordingly, the multiple polymerase III promoter expression constructs of the invention will include an appropriate oligo (dT) termination signal, i.e., a sequence of 4, 5, 6 or more Ts, operably linked 3' to each RNA Pol III promoter in the DNA coding strand.

These vectors can be used to transcribe the desired RNA molecule in the cell according to this invention. Vectors may be desirably designed to utilize an endogenous mitochondrial RNA polymerase (e.g., human mitochondrial RNA polymerase, in which case such vectors may utilize the corresponding human mitochondrial promoter). Mitochondrial polymerases may be used to generate capped (through expression of a capping enzyme) or uncapped messages *in vivo*. RNA pol I, RNA pol II, and RNA pol III transcripts may also be generated *in vivo*. Such RNAs may be capped or not, and if desired, cytoplasmic capping may be accomplished by various means including use of a capping enzyme such as a vaccinia capping enzyme or an alphavirus capping enzyme. However, all pol II transcripts are capped. The DNA vector is designed to contain one of the promoters or multiple promoters in combination (mitochondrial, RNA pol I, pol II, or pol III, or viral, bacterial or bacteriophage promoters along with the cognate polymerases). Preferably, where the promoter is RNA pol II, the sequence encoding the RNA molecule has an open reading frame greater than about 300 nts and must follow the rules of design to prevent nonsense-mediated degradation in the nucleus. Such plasmids or vectors can include plasmid sequences from bacteria, viruses or phages.

Such vectors include chromosomal, episomal and virus-derived vectors, e.g., vectors derived from bacterial plasmids, bacteriophages, yeast episomes, yeast chromosomal elements, and viruses, vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, cosmids and phagemids.

Replace the section beginning at page 31, line 26, and ending at page 34, line 29, with the following amended section:

The term "*in vivo*" is intended to include any system wherein the cellular DNA or RNA replication machinery is intact, preferably within intact living cells, including tissue culture systems, tissue explants, and within single cell or multicellular living organisms.

By "multiple sequitope dsRNA" or "multisequitope dsRNA" or "multiple epitope dsRNA" is meant an RNA molecule that has segments derived from multiple target nucleic acids or that has non-contiguous segments from the same target nucleic acid. For example, the multiple sequitope dsRNA may have segments derived from (i) sequences representing multiple genes of a single organism; (ii) sequences representing one or more genes from a variety of different organisms; and/or (iii) sequences representing different regions of a particular gene (e.g., one or more sequences from a promoter and one or more sequences from an mRNA. Desirably, each segment has substantial sequence identity to the corresponding region of a target nucleic acid. In various desirable embodiments, a segment with substantial sequence identity to the target nucleic acid is at least 19, 20, 21, 22, 23, 24, 25, 26, 27, 30, 40, 50, 100, 200, 500, 750, or more basepairs in length. In desirable embodiments, the multiple epitope dsRNA inhibits the expression of at least 2, 4, 6, 8, 10, 15, 20, or more target genes by at least 20, 40, 60, 80, 90, 95, or 100%. In some embodiments, the multiple epitope dsRNA has non-contiguous segments from the same target gene or from the same target polynucleotide that may or may not be in the naturally occurring 5' to 3' order of the segments, and the dsRNA inhibits the expression of the target nucleic acid by at least 50, 100, 200, 500, or 1000% more than a dsRNA with only one of the segments.

By "sequitope" is meant a contiguous sequence of double-stranded polyribonucleotides that can associate with and activate RISC (RNA-induced silencing complex), usually a contiguous sequence of between 19 and 27 basepairs, inclusive. Sequences comprising at least one sequitope from within one or more of the conserved HBV and/or HCV nucleotide sequences identified here may be utilized for dsRNA mediated gene silencing as taught herein.

Multiple-epitope/ multiple-sequitope dsRNAs the advantages of a multiple-epitope or multisequitope double-stranded RNA approach as taught in USSN 60/419,532, filed 18-Oct-2002 and PCT/US2003/033466, filed 20-Oct-2003, are applicable to utilization of the conserved HBV and/or HCV sequences of the invention. Because a singular species of dsRNA can simultaneously silence many target genes (e.g., genes from multiple pathogens, multiple genes or sequences from a single pathogen, or genes associated with multiple diseases), a multiple epitope dsRNA can be used for many different indications in the same subject or used for a subset of indications in one subject and another subset of indications in another subject. For such applications, the ability to express long dsRNA molecules (e.g., dsRNA molecules with sequences from multiple genes) without invoking the dsRNA stress response is highly desirable. For example, by using a series of sequences, each, e.g., as short as 19-21 nucleotides, desirably 100 to 600 nucleotides, or easily up to 1, 2, 3, 4, 5, or more kilobases such that the total length of such sequences is within the maximum capacity of the selected plasmid (e.g., 20 kilobases in length), a single such pharmaceutical composition can provide protection against a large number of pathogens and/or toxins at a relatively low cost and low toxicity, e.g., HBV, HCV, HIV, etc.

The use of multiple epitopes or sequitopes derived from one or more genes and/or different overlapping and/or non-contiguous sequences of the same polynucleotide or gene from multiple strains and/or variants of a highly variable or rapidly mutating pathogen such as HBV and/or HCV can also be very advantageous. For example, a singular dsRNA species that recognizes and targets multiple strains and/or variants of HBV and/or HCV can be used as a universal treatment or vaccine for the various strains/variants of HBV and/or HCV.

The ability to silence multiple genes of a particular pathogen such as HBV and/or HCV prevents the selection of, in this case, HBV and/or HCV "escape

mutants." In contrast, typical small molecule treatment or vaccine therapy that only targets one gene or protein results in the selection of pathogens that have sustained mutations in the target gene or protein and the pathogen thus becomes resistant to the therapy. By simultaneously targeting a number of genes or sequences of the pathogen and/or extensive regions of the pathogen using the multiple epitope approach of the present invention, the emergence of such "escape mutants" is effectively precluded.

For example, it is considered particularly advantageous to include a mixture of sequences from both HCV SEQ ID NO:11 and SEQ ID NO:12, and SEQ ID NO: 27, i.e., one or more sequences (e.g, each at least 19, 20, 21, 22, 23, 24, 25, 26, 27 to 29 contiguous nucleotides) from HCV SEQ ID NO:11 together with one or more sequences (e.g, each at least 19, 20, 21, 22, 23, 24, 25, 26, 27 to 29 contiguous nucleotides) from HCV SEQ ID NO:12 and from SEQ ID NO: 27 , either in a single dsRNA molecule, an admixture of dsRNA molecules, or through concomitant administration of such molecules to a patient (or by administering one or more dsRNA expression constructs which produce such dsRNA molecules intracellularly), in order to decrease the ability of the virus to generate viable escape mutants. Similarly, it would be advantageous to provide a mixture of dsRNA molecules comprising a number of the conserved HBV sequences, in some cases in combination with one or more of the conserved HCV sequences of the invention.

Similarly, it may be desirable to use sequences from two or more of HBV SEQ ID NO:1, SEQ ID NO:2, AND SEQ ID NO:3, either in a single dsRNA construct, an admixture of constructs, or through concomitant administration of such constructs (or dsRNA expression constructs which produce such dsRNA molecules) to a patient. SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 map to the HBV surface antigen genes. Due to the overlapping nature of the HBV mRNAs, the following mRNAs would be targeted by one of more of these sequences: Surface Ag (sAg) mRNAs, precore, core and polymerase mRNAs. However, since sAg mRNAs are the most abundant, it is more likely that these mRNAs will be targeted if the gene-silencing machinery is saturable. It is possible, however, that all listed mRNAs will be targeted. Reduction of surface Ag is desirable for several reasons: a) surface Ag is needed for assembly of infectious virions; b) overexpression of Surface Ag during infection is thought to contribute to immune anergy that occurs during chronic HBV infection;

and c) the expression of HBVsAg in the livers of infected individuals (even in the absence of virus, i.e., from integrated sAg sequences into the host genome) induces hepatitis. Therefore, reduction of sAg is likely to decrease viral titers, overcome immune anergy and decrease/prevent hepatitis.

Replace the section beginning at page 35, line 3, and ending at page 42, line 2, with the following amended section:

HBV SEQ ID NO:5 through SEQ ID NO:8 map to the polymerase gene. Effector RNAs are predicted to target only precore/core and polymerase transcripts. There should be no competition with sAg or X mRNAs. Polymerase is needed for the synthesis of viral genomes and therefore viral particle titer is expected to decrease as polymerase is decreased.

HBV SEQ ID NO:9 maps to the X gene. Due to the terminal redundancy of all the HBV mRNAs, these effector RNAs have the potential to target all of the HBV viral mRNAs. X protein has many ascribed (non proven) functions. Evidence is emerging, however, that X-gene expression is associated with hepatocellular carcinogenesis, in part related to promotion of detachment and migration of cells out of the primary tumor site. Since the X gene is often found in integrated HBV sequences in individuals with and without active hepatitis, down-regulation of X gene expression is predicted to ameliorate disease, including the incidence of hepatocellular carcinoma.

In general, the more sequences or sequitopes from the different identified sequences that are used (e.g., from SEQ ID NO:1, SEQ ID NO:2, and/or SEQ ID NO:3, plus sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10), the less likely a virus will be able to generate viable escape mutants. Also, the more different mRNAs that can be targeted, the more significant will be the drops in viral titer and disease amelioration.

Desirable combinations for multiepitope or multisequitope dsRNA expression constructs or dsRNA effector molecules, an admixture of dsRNA expression constructs or dsRNA effector molecules , or the concomitant administration of different dsRNA expression constructs or dsRNA effector molecules include the following: Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus

sequences from SEQ ID NO:4; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:5; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:6; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:7; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:8; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:9; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:10; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:4 and SEQ ID NO:5; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:4 and SEQ ID NO:6; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:4 and SEQ ID NO:7; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:4 and SEQ ID NO:8; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:4 and SEQ ID NO:9; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:4 and SEQ ID NO:10; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:5 and SEQ ID NO:6; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:5 and SEQ ID NO:7; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:5 and SEQ ID NO:8; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:5 and SEQ ID NO:9; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:5 and SEQ ID NO:10; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:6 and SEQ ID NO:7; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:6 and SEQ ID NO:8; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:6 and SEQ ID NO:9; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:6 and SEQ ID NO:10; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:7 and SEQ ID NO:8; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:7 and SEQ ID

NO:9; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:7 and SEQ ID NO:10; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:8 and SEQ ID NO:9; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:8 and SEQ ID NO:10; Sequences from SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 plus sequences from SEQ ID NO:9 and SEQ ID NO:10; Sequences from SEQ ID NO:4 and SEQ ID NO:5; Sequences from SEQ ID NO:4 and SEQ ID NO:6; Sequences from SEQ ID NO:4 and SEQ ID NO:7; Sequences from SEQ ID NO:4 and SEQ ID NO:8; Sequences from SEQ ID NO:4 and SEQ ID NO:9; Sequences from SEQ ID NO:4 and SEQ ID NO:10; Sequences from SEQ ID NO:5 and SEQ ID NO:6; Sequences from SEQ ID NO:5 and SEQ ID NO:7; Sequences from SEQ ID NO:5 and SEQ ID NO:8; Sequences from SEQ ID NO:5 and SEQ ID NO:9; Sequences from SEQ ID NO:5 and SEQ ID NO:10; Sequences from SEQ ID NO:6 and SEQ ID NO:7; Sequences from SEQ ID NO:6 and SEQ ID NO:8; Sequences from SEQ ID NO:6 and SEQ ID NO:9; Sequences from SEQ ID NO:6 and SEQ ID NO:10; Sequences from SEQ ID NO:7 and SEQ ID NO:8; Sequences from SEQ ID NO:7 and SEQ ID NO:9; Sequences from SEQ ID NO:7 and SEQ ID NO:10; Sequences from SEQ ID NO:8 and SEQ ID NO:9; Sequences from SEQ ID NO:8 and SEQ ID NO:10; Sequences from SEQ ID NO:9 and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5; and SEQ ID NO:6; Sequences from SEQ ID NO:4, SEQ ID NO:5; and SEQ ID NO:7; Sequences from SEQ ID NO:4, SEQ ID NO:5; and SEQ ID NO:8; Sequences from SEQ ID NO:4, SEQ ID NO:5; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:5; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:6; and SEQ ID NO:7; Sequences from SEQ ID NO:4, SEQ ID NO:6; and SEQ ID NO:8; Sequences from SEQ ID NO:4, SEQ ID NO:6; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:6; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:7; and SEQ ID NO:8; Sequences from SEQ ID NO:4, SEQ ID NO:7; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:7; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6; and SEQ ID NO:7; Sequences from SEQ ID NO:5, SEQ ID NO:6; and SEQ ID NO:8;

Sequences from SEQ ID NO:5, SEQ ID NO:6; and SEQ ID NO:9; Sequences from SEQ ID NO:5, SEQ ID NO:6; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:7; and SEQ ID NO:8; Sequences from SEQ ID NO:5, SEQ ID NO:7; and SEQ ID NO:9; Sequences from SEQ ID NO:5, SEQ ID NO:7; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:5, SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:6, SEQ ID NO:7; and SEQ ID NO:8; Sequences from SEQ ID NO:6, SEQ ID NO:7; and SEQ ID NO:9; Sequences from SEQ ID NO:6, SEQ ID NO:7; and SEQ ID NO:10; Sequences from SEQ ID NO:6, SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:6, SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:6, SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:7, SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:7, SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:7, SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:8, SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:6; and SEQ ID NO:7; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:8; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:6; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:7; and SEQ ID NO:8; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:7; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:7; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:6; SEQ ID NO:7; and SEQ ID NO:8; Sequences from SEQ ID NO:4, SEQ ID NO:6; SEQ ID NO:7; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:6; SEQ ID NO:7; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:7; SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:7; SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:7; SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8; Sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:9; Sequences from SEQ ID NO:5,

SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6; SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:5, SEQ ID NO:6; SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6; SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:7; SEQ ID NO:8; and SEQ ID NO:9; Sequences from SEQ ID NO:5, SEQ ID NO:7; SEQ ID NO:8; and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:7; SEQ ID NO:9; and SEQ ID NO:10; Sequences from SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; Sequences from SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:10; Sequences from SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; Sequences from SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; Sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; Sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; and Sequences from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10. Preferred in some aspects are sequences from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, including combinations of sequitopes from SEQ ID NO:5, plus SEQ ID NO:6, plus SEQ ID NO:7, plus SEQ ID NO:8.

In another embodiment, combinations of sequitopes at least 19 contiguous base pairs in length and longer sequences from within any of the aforementioned sequences (e.g., SEQ ID NO:1 through SEQ ID NO:12) may be utilized either in a single dsRNA expression construct or dsRNA effector molecule, an admixture of dsRNA expression constructs or dsRNA effector molecules or through concomitant administration of such dsRNA expression constructs or dsRNA effector molecules to a patient. By a sequence of "at least 19 contiguous base pairs in length" is meant that a sequence or sequitope of at 19 contiguous bases in length is present in double-stranded conformation, or within a double-stranded RNA effector molecule.

As discussed elsewhere herein, a particularly preferred embodiment of the invention utilizes dsRNA expression constructs or vectors to achieve endogenous delivery of the dsRNAs of the invention, especially the multiple different sequences described above. These dsRNAs may be provided e.g., on the same cistron of an expression construct such as a plasmid, on different cistrons of an expression construct, or on different expression constructs or plasmids, e.g., one or more plasmids and/or one or more vectors, including viral vectors. The combination of different dsRNA effector molecules such as shRNA effector molecules may be provided to a mammalian cell by in vivo expression from a single expression construct such as a plasmid, with each dsRNA effector molecule transcribed from a different expression cassette driven by a different promoter, e.g., an RNA

polymerase I promoter and/or an RNA polymerase III promoter, e.g., a type 3 RNA polymerase III promoter such as U6, H1, 7SK, or MRP. In some embodiments, each such different expression cassette may contain a different RNA polymerase III promoter, which may be the same or different, and an RNA polymerase III termination sequence. In another embodiment, a combination of different dsRNA effector molecules such as shRNA effector molecules may be provided to a mammalian cell by in vivo expression from a single expression construct such as a plasmid or a viral vector which comprise an expression cassette comprising multiple different promoters, e.g., an RNA polymerase I promoter and/or an RNA polymerase III promoter, e.g., a type 3 RNA polymerase III promoter such as U6, H1, 7SK, or MRP, and wherein each of such promoters transcribes a different dsRNA effector molecule. Such multiple different dsRNA effector sequences may also be provided to an in vivo mammalian cell exogenously, in any different mixture of one or more dsRNA structures, duplexes and/or harpins, and/or in combination with one or more endogenously expressed dsRNA structures.

Desirable methods of administration of nucleic acids The DNA and/or RNA constructs, e.g., dsRNA effector molecules, of the invention may be administered to the host cell/tissue/organism as "naked" DNA, RNA, or DNA/RNA, formulated in a pharmaceutical vehicle without any transfection promoting agent. More efficient delivery may be achieved as known to those of skill in the art of DNA and RNA delivery, using e.g., such polynucleotide transfection facilitating agents known to those of skill in the art of RNA and/or DNA delivery. The following are exemplary agents: cationic amphiphiles including local anesthetics such as bupivacaine, cationic lipids, liposomes or lipidic particles, polycations such as polylysine, branched, three-dimensional polycations such as dendrimers, carbohydrates, detergents, or surfactants, including benzylammonium surfactants such as benzalkonium chloride. Non-exclusive examples of such facilitating agents or co-agents useful in this invention are described in U.S. Patent numbers 5,593,972; 5,703,055; 5,739,118; 5,837,533; 5,962,482; 6,127,170; 6,379,965; and 6,482,804; and International Patent Application No. PCT/US98/22841; the teaching of which is hereby incorporated by reference. U.S. Patents numbers 5,824,538; 5,643,771; and 5,877,159 (incorporated herein by reference) teach delivery of a composition other

than a polynucleotide composition, e.g., a transfected donor cell or a bacterium containing the dsRNA-encoding compositions of the invention.

Replace the paragraph beginning at page 43, line 20, with the following amended paragraph:

In general, targeting for selective delivery of the dsRNA constructs of the invention to hepatocytes is preferred. Targeting to hepatocytes may be achieved by coupling to ligands for hepatocyte-specific receptors. For example, asialo-orosomucoid, (poly)L-lysine-asialo-orosomucoid, or any other ligands of the hepatic asialoglycoprotein receptor (Spiess, Biochemistry 29(43):10009-10018, 1990; Wu et al., J. Biol. Chem. 267(18):12436-12439, 1992; Wu et al., Biotherapy 3:87-95, 1991). Similarly, the oligonucleotides may be targeted to hepatocytes by being conjugated to monoclonal antibodies that specifically bind to hepatocyte-specific receptors. Oligonucleotides may also be targeted to hepatocytes using specific vectors, as described below.

Replace the paragraph beginning at page 44, line 6, with the following amended paragraph:

The dsRNA oligonucleotides of the invention may be provided exogenously to a target hepatocyte, e.g., prepared outside the cell and delivered into a mammalian hepatocyte. Alternatively, a dsRNA may be produced within the target cell by transcription of a nucleic acid molecule comprising a promoter sequence operably linked to a sequence encoding the dsRNA. In this method, the nucleic acid molecule is contained within a non-replicating linear or circular DNA or RNA molecule, or is contained within an autonomously replicating plasmid or viral vector, or is integrated into the host genome. Any vector that can transfect a hepatocyte may be used in the methods of the invention. Preferred vectors are viral vectors, including those derived from replication-defective hepatitis viruses (e.g., HBV and HCV), retroviruses (see, e.g., WO89/07136; Rosenberg et al., N. Eng. J. Med. 323(9):570-578, 1990), adenovirus (see, e.g., Morsey et al., J. Cell. Biochem., Supp. 17E, 1993; Graham et al., in Murray, ed., Methods in Molecular Biology: Gene Transfer and Expression

Protocols. Vol. 7, Clifton, N.J.: the Human Press 1991: 109-128), adeno-associated virus (Kotin et al., Proc. Natl. Acad. Sci. USA 87:2211-2215, 1990), replication defective herpes simplex viruses (HSV; Lu et al., Abstract, page 66, Abstracts of the Meeting on Gene Therapy, Sep. 22-26, 1992, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), and any modified versions of these vectors. Methods for constructing expression vectors are well known in the art (see, e.g., Molecular Cloning: A Laboratory Manual, Sambrook et al., eds., Cold Spring Harbor Laboratory, 2nd Edition, Cold Spring Harbor, N.Y., 1989).

Add, before the paragraph beginning at page 45, line 3, the following new section:

Upon assembly of a recombinant DNA plasmid dsRNA expression vector on the invention, bacteria are used as "factories" to produce large quantities of the final vector. The E.coli bacterium is frequently used for plasmid fermentation, and it may be advantageous to employ for this purpose E. coli strains having a reduced genome as described in, e.g., Blattner et al., Published U.S. Patent Application No. 2005/0032225, the teaching of which is incorporated herein by reference. The vector manufactured in this manner, isolated and purified according to methods known in the art, can be introduced into living cells with a variety of methods, collectively known as "transfection", including the methods and compositions described above. Once inside the cell, the promoter elements are recognized by the cellular machinery available for gene transcription and the RNA effector molecules, e.g., shRNAs, will be produced.

Other bacterial strains that may be advantageous for propagating a plasmid expression vector of the invention include the E. coli GT116 Competent Cells available commercially from InvivoGen, San Diego, CA. GT116 is a sbcCD deletion strain specifically engineered to support the growth of plasmid DNAs carrying hairpin structures, such as the plasmids of the invention engineered to express one or more dsRNA effector molecules which are hairpin RNAs. Hairpin structures are known to be unstable in E. coli due to their elimination by a protein complex called SbcCD that recognizes and cleaves hairpins (Connelly et al., Proc. Natl. Acad. Sci. USA 95:7969-74 (1998)). The sbcCD and sbcD genes are deleted in E. coli GT116,

which improves its utility for cloning plasmids with hairpin or other palindrome-containing structures.

Replace the paragraph beginning at page 45, line 26, with the following amended paragraph:

dsRNA expression vectors may include promoters for RNA polymerase I, RNA polymerase II including but not limited to HCMV, SCMV, MCMV, RSV, EF2a, TK and other HSV promoters such as ICP6, ICP4 and ICP0 promoters, HBV pregenomic promoter, RNA pol III promoter, especially type 3 RNA polymerase III promoters, including but not limited to 7SK, U6, and H1, and tRNA promoters, as well as mitochondrial light and heavy strand promoters. Desirably, the dsRNA expression vector comprises at least one RNA polymerase II promoter, for example, a human CMV-immediate early promoter (HCMV-IE) or a simian CMV (SCMV) promoter, at least one RNA polymerase I promoter, or at least one RNA polymerase III promoter. The promoter may also be a T7 promoter, in which case, the cell further comprises T7 RNA polymerase. Alternatively, the promoter may be an SP6 promoter, in which case, the cell further comprises SP6 RNA polymerase. The promoter may also be one convergent T7 promoter and one convergent SP6 RNA promoter. A cell may be made to contain T7 or SP6 polymerase by transforming the cell with a T7 polymerase or an SP6 polymerase expression plasmid, respectively. In some embodiments, a T7 promoter or a RNA polymerase III promoter is operably linked to a nucleic acid that encodes a short dsRNA (e.g., a dsRNA that is less than 200, 150, 100, 75, 50, or 25 basepairs in length). In other embodiments, the promoter is a mitochondrial promoter that allows cytoplasmic transcription of the nucleic acid in the vector (see, for example, the mitochondrial promoters described in WO 00/63364, filed April 19, 2000, and in WO/US2002/00543, filed 9-Jan-2001). Alternatively, the promoter is an inducible promoter, such as a *lac* (Cronin et al. Genes & Development 15: 1506-1517, 2001*), ara* (Khlebnikov et al., J Bacteriol. 2000 Dec;182(24):7029-34), ecdysone (Rheogene website), RU48 (mefepristone) (corticosteroid antagonist) (Wang XJ, Liefer KM, Tsai S, O'Malley BW, Roop DR, Proc Natl Acad Sci U S A. 1999 Jul 20;96(15):8483-8), or *tet* promoter (Rendal et al., Hum Gene Ther. 2002;13(2):335-42 and Larnartina et al., Hum Gene Ther.

2002;13(2):199-210) or a promoter disclosed in WO 00/63364, filed April 19, 2000. Also useful in the methods and compositions of the invention are the structural and chimeric promoters taught in USSN 60/464,434, filed 22-Apr-2003. See also the promoter systems taught in Pachuk, C., and Satishchandran, C., "Multiple-Compartment Eurkaryotic Expression Systems," U.S. Provisional Application No. 60/497,304, filed 22-Aug-2003, which are considered particularly desirable in the methods and compositions of the invention.

Replace the paragraph beginning at page 49, line 17, with the following amended paragraph:

Routes of administration include, but are not limited to, intramuscular, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterially, intraoccularly and oral as well as transdermally or by inhalation or suppository. Preferred routes of administration include intravenous, intramuscular, oral, intraperitoneal, intradermal, intraarterial and subcutaneous injection.

Add, before the paragraph beginning at page 50, line 3, the following new section:

Targeted transfection of hepatocytes *in vivo* for delivery of dsRNAs against HBV and/or HCV may be accomplished through IV injection with a composition comprising a DNA or RNA expression vector as described herein complexed with a mixture (e.g., a 35%/65% ratio) of a lactosyl spermine (mono or trilactosylated) and cholesteryl spermine (containing spermine to DNA at a charge ratio of 0.8). Such compositions are especially useful for pharmaceutical applications and may readily be formulated in a suitable sterile, non-pyrogenic vehicle, e.g., buffered saline for injection, for parenteral administration, e.g., IV (including IV infusion), IM, SC, and for intraperitoneal administration, as well as for aerosolized formulations for pulmonary delivery via inhalation. In certain formulations, a DNA expression construct of the invention may be complexed with an endosomolytic spermine such cholesteryl spermine alone, without a targeting spermine; some routes of administration, such as intraperitoneal injection or infusion, may achieve effective hepatic delivery and

transfection of a DNA construct of the invention, and expression of RNA effector molecules, e.g., multiple dsRNA hairpins effective against HBV and/or HCV.

Intraperitoneal administration (e.g., ultrasound guided intraperitoneal injection) of a sterile pharmaceutical composition comprising dsRNA effector molecules and/or dsRNA expression constructs which provide dsRNA effector molecules against HBV and/or HCV in a specially formulated delivery vehicle may be an advantageous route of delivery to promote uptake by liver cells, including hepatocytes. In some compositions the dsRNA expression construct may be complexed with an appropriate transfection enhancing agent, e.g., with a mixture of a lactosyl spermine (mono or trilactosylated) and cholesteryl spermine, or in other compositions with an endosomolytic spermine such cholesteryl spermine alone, without a targeting spermine. The volume, concentration, and formulation of the pharmaceutical composition as well as the dosage regimen may be tailored specifically to maximize cellular delivery while minimizing toxicity such as an inflammatory response. E.g, relatively large volumes (5, 10, 20, 50 ml or more) with corresponding low concentrations of active ingredients, as well as the inclusion of an anti-inflammatory compound such as a corticosteroid, may be utilized if desired. Formulations as known to those of skill in the art of pharmaceutics may also be utilized to provide sustained release of dsRNA effector molecules and/or dsRNA expression constructs of the invention.

dsRNAs or dsRNA expression constructs may be administered by means including, but not limited to, traditional syringes, needleless injection devices, or "microprojectile bombardment gene guns". Intraperitoneal injection may be accomplished, e.g., with a traditional syringe, with placement of the needle advantageously guided by ultrasound or a similar technique. Alternatively, the dsRNA and/or dsRNA expression construct may be introduced by various means into cells that are removed from the individual. Such means include, for example, *ex vivo* transfection, electroporation, microinjection and microprojectile bombardment. After the gene construct is taken up by the cells, they are reimplanted into the individual. It is contemplated that otherwise non-immunogenic cells that have gene constructs incorporated therein can be implanted into the individual even if the host cells were originally taken from another individual.

Replace the paragraph beginning at page 51, line 3, with the following amended paragraph:

For administration in an intact animal, e.g., a human subject infected with HBV and/or HCV, between 1 mg and 100 mg, typically between 1 mg and 10 mg, between 10 ng and 50 µg, between 50 ng and 100 ng, or between 100 ng and 5 µg of dsRNA or DNA encoding one or more dsRNA effector molecules is used. In desirable embodiments, approximately 10 µg of a DNA or 5 µg of dsRNA is administered to the animal. In a desirable embodiment, a pharmaceutical composition for parenteral administration is prepared containing 10 mg of a plasmid dsRNA expression construct of the invention (in some formulations complexed with an appropriate transfection facilitating agent such as cholesteryl spermine or a mixture of cholesteryl spermine/trilactosyl spermine) in 25 ml of a suitable sterile vehicle for injection such as Normal Saline Injection, D5W, D5%/0.45% NaCl, D5%/0.2% NaCl, etc., and injected intraperitoneally over 5 to 10 minutes, with needle placement guided by untrasound or a similar technology. Administration may be repeated periodically, e.g., weekly or monthly, as required. With respect to the methods of the invention, it is not intended that the administration of dsRNA or DNA encoding dsRNA to cells or animals be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration sufficient to provide a dose adequate to inhibit gene expression, prevent a disease, or treat a disease.

Add, before the paragraph beginning at page 51, line 18, the following new section:

Therapeutic Advantages of the Invention as it Relates to Worldwide Disease Incidence and Viral Variability

The mutability of the hepatitis C virus genome, and to a lesser but significant extent, the hepatitis B virus genome, has been described above as presenting challenges to the design of nucleic acid based therapeutics against these viral agents. The inventors have painstakingly aligned thousands of individual HCV and HBV sequences, originally deriving from thousands of human viral isolates from widely divergent geographic areas worldwide. In doing so, the instant invention has

identified and specified preferred sequences which are utilized singly and in combination in dsRNA effector molecules which target the least mutable regions of the genome of HCV and/or HBV.

The two-fold rationale for this has been discussed above, primarily in terms of ensuring that during the course of infection of a patient with HBV or HCV, the therapeutic of the invention will remain potent against the virus even as it mutates during the course of disease in a given patient. However, the second part of this rationale for deriving and using highly conserved sequences for design of dsRNA-based therapeutic applications, is that this also increases the certainty that the therapeutic dsRNA effector molecules of the invention, particularly the methods and compositions of the invention which utilize combinations of highly conserved sequences in dsRNA effector molecules against HBV and/or HCV, will be able to treat the viral infection present in individuals from a global variety of different ancestries, genetic makeup, and geographical distribution, which are known to manifest in clusters of viral variants based on such factors. Thus, a key feature of the therapeutic utility and novelty of this invention lies in the method of derivation of the preferred sequences and embodiments, and not simply in the demonstration that any particular HCV or HBV dsRNA sequence can inhibit viral replication of one or a few chosen viral isolates (or their cognate replicons) in a laboratory experiment, i.e., in a cell line or animal model, not necessarily reflective of the broad diversity of the HBV and/or HCV virus worldwide, or even in a particular infected individual over the course of infection.

For example, the hepatitis B virus has four subtypes of surface antigen, namely adw, ayw, adr and ayr. While lamivudine is considered an effective therapy for chronic Hepatitis B, a recent study of HBV resistance demonstrated a 20-fold increase in resistance in the adw subtype, compared to the ayw subtype. B Zollner et al. "20-fold Increase in Risk of Lamivudine [Epivir HBV] Resistance in Hepatitis B Virus Subtype adw"; *The Lancet*. 2001; 357: 934-935. In contrast to such conventional antiviral agents, the dsRNA agents of the invention, (e.g., dsRNA effector molecules and expression constructs of the invention, especially when used in combination as taught herein) which utilize HBV and/or HCV sequences highly conserved across such geographical genetic variants are expected to exhibit highly advantageous antiviral activity.

Similarly, HCV is also known for having a wide range of geographically divergent viral genotypes, subtypes, quasispecies, with the following current general global patterns of genotypes and subtypes:

1a - mostly found in North & South America; also common in Australia
1b - mostly found in Europe and Asia.
2a - is the most common genotype 2 in Japan and China.
2b - is the most common genotype 2 in the US and Northern Europe.
2c - the most common genotype 2 in Western and Southern Europe.
3a - highly prevalent here in Australia (40% of cases) and South Asia.
4a - highly prevalent in Egypt
4c - highly prevalent in Central Africa
5a - highly prevalent only in South Africa
6a - restricted to Hong Kong, Macau and Vietnam
7a and 7b - common in Thailand
8a, 8b & 9a - prevalent in Vietnam
10a & 11a - found in Indonesia

Accordingly, the highly conserved sequences of the invention, which are expected to be conserved among most if not all of these divergent HCV genotypes and subtypes worldwide, including 1a, 1b, 2a, 2b, and 2c, are considered highly effective therapeutics agents, e.g., when utilized as dsRNA effector molecules, especially combinations thereof, and dsRNA expression vectors capable of expressing such dsRNAs.

Replace the section beginning at page 52, line 25, and ending at page 53, line 21, with the following amended section:

Using this model, cells were co-transfected with the infectious molecular clone of HBV and various eiRNA constructs (dsRNA expression constructs). The cells were then monitored for loss of HBV expression and replication as described below. Details on the vector and encoded RNAs used in this experiment are provided at the end of this example.

Experiment 1:

The following is an example of an experiment that was performed using eiRNA vectors (dsRNA expression vectors) encoding sequences derived from GenBank accession number V01460. HBV sequences in these described eiRNA vectors were

highly conserved sequences identified as described elsewhere herein, which also exhibited activity as siRNAs (See, Pachuk, C., "Methods and Constructs for Evaluation of RNAi targets and Effector Molecules," PCT/US2004/005065, filed 25-Feb-2004). The particular eiRNA backbone vector used for this experiment was a proprietary vector containing a U6 promoter to drive expression of the encoded RNAs. Each vector encoded only one short hairpin RNA (shRNA). The shRNA coding sequence was followed by an RNA pol III termination sequence. Sequences of the U6 promoter, RNA pol III termination signal, and encoded shRNAs are all shown at the end of the example. Similar vectors containing U6 promoters and RNA pol III termination signals are commercially available such as the "siLentGene-2 Cloning Systems" vector from Promega, Inc., Madison, Wis. One of ordinary skill in the art can also create them according to the information provided herein. It is expected that similar results would also be obtained using other expression and promoter systems especially those vectors with RNA pol III promoters that are not U6, for example H1 promoters or 7SK promoters.

Replace Table 1 at page 56 with the following amended Table 1:

Table 1

HBV-AYW coordinates*	SEQ ID NO		Maps within SEQ ID NO
788-808	14	CGTCTGCGAGGCGAGGGAGTTAGAGAACTTAACTCCCTCGCCTCGCAGACG	5, 6, 7, or 8
807-827	15	TTCTTCTTCTAGGGGACCTGCAGAGAACTTGCAGGTCCCCTAGAAGAAGAA	5, 6, 7, or 8
1291-1311	16	AAGCCACCCAAGGCACAGCTTAGAGAACTTAAGCTGTGCCTTGGGTGGCTT	4
1299-1319	17	CAAGGCACAGCTTGGAGGCTTAGAGAACTTAAGCCTCCAAGCTGTGCCTTG	4
1737-1757	18	GGATTCAGCGCCGACGGGACGAGAGAACTTCGTCCCGTCGGCGCTGAATCC	10
1907-1927	19	TTCCGCAGTATGGATCGGCAGAGAGAACTTCTGCCGATCCATACTGCGGAA	3
1912-1932	20	CAGTATGGATCGGCAGAGGAGAGAGAACTTCTCCTCTGCCGATCCATACTG	3
1943-1963	21	TCCACGCATGCGCTGATGGCCAGAGAACTTGGCCATCAGCGCATGCGTGGA	3
1991-2011	22	TGCGTCAGCAAACACTTGGCAAGAGAACTTTGCCAAGTGTTTGCTGACGCA	3
2791-2811	23	AAAACGCCGCAGACACATCCAAGAGAACTTTGGATGTGTCTGCGGCGTTTT	2
2791-2811mut	24	AAAACACCACACACGCATCCAAGAGAACTTTGGATGCGTGTGTGGTGTTTT	2
2912-2932	25	TTGAGAGAAGTCCACCACGAGAGAGAACTTCTCGTGGTGGACTTCTCTCAA	1
2919-2939	26	AAGTCCACCACGAGTCTAGACAGAGAACTTGTCTAGACTCGTGGTGGACTT	1
799-779	49	GCCTCGCAGACGAAGGTCTCAAGAGAACTTTGAGACCTTCGTCTGCGAGGC	

*nucleotide coordinates refer to Genbank accession number V01460.

Add, before the paragraph beginning at page 57, line 5, the following new section:

The HBV sequitopes of Table 1 (without their respective complement sequence and without the "loop" or linker sequence utilized in a "hairpin" or stem-loop dsRNA effector molecule) are shown in Table 1A below. Each such HBV sequitope, together with its complementary sequence, optionally with an appropriate loop or linker sequence as taught herein, may be utilized in a dsRNA effector molecule of the invention (e.g., a duplex dsRNA or hairpin dsRNA effector molecule, or encoded within a dsRNA expression vector). In one aspect, multiple (e.g., 2, 3, 4, 5, 6, or more) of such dsRNA short hairpin effector molecules are encoded in a single expression vector, e.g., a plasmid expression vector, each under the control of a different promoter, e.g., a polymerase III promoter, as described elsewhere herein.

TABLE 1A

HBV-AYW coordinates *Genbank accession number V01460**	SEQ ID NO	Conserved HBV Sequitope	Maps within SEQ ID NO
788-808	50	CGTCTGCGAGGCGAGGGAGTT	5, 6, 7, or 8
807-827	51	TTCTTCTTCTAGGGGACCTGC	5, 6, 7, or 8
1291-1311	52	AAGCCACCCAAGGCACAGCTT	4
1299-1319	53	CAAGGCACAGCTTGGAGGCTT	4
1737-1757	54	GGATTCAGCGCCGACGGGACG	10
1907-1927	55	TTCCGCAGTATGGATCGGCAG	3
1912-1932	56	CAGTATGGATCGGCAGAGGAG	3
1943-1963	57	TCCACGCATGCGCTGATGGCC	3
1991-2011	58	TGCGTCAGCAAACACTTGGCA	3
2791-2811	59	AAAACGCCGCAGACACATCCA	2
2912-2932	60	TTGAGAGAAGTCCACCACGAG	1
2919-2939	61	AAGTCCACCACGAGTCTAGAC	1
799 - 779	62	GCCTCGCAGACGAAGGTCTCA	

Add, before the paragraph beginning at page 66, line 4, the following new section:

A Four-Promoter RNA polymerase III-based expression construct for production of shRNAs which reduce Hepatitis B RNA production and replication.

As described in more detail in PCT/US05/29976 filed 23-August-2005, a plasmid expression vector, pHB4, containing 4 polymerase III promoter short hairpin dsRNA expression cassettes was constructed. Each expression cassette included a polymerase III promoter operably linked to a sequence encoding a short hairpin dsRNA, and a polymerase III termination sequence. The polymerase III promoters were U6, 7SK, and two copies of a 7SK sequence variant promoter, designated 7SK-4A. Each short hairpin dsRNA sequence comprised a double-stranded stem region homologous and complementary to a highly conserved HBV sequence as taught herein. The four dsRNA effector molecules comprised the sequences of SEQ ID NO: 49; SEQ ID NO: 23; SEQ ID NO:19; and SEQ ID NO: 18; which comprise, respectively; the sequences of SEQ ID NO: 62; SEQ ID NO: 59; SEQ ID NO:55; and SEQ ID NO: 54. As described in more detail in Example 1 of PCT/US05/29976, however, the sequence encoding the dsRNA hairpin effector molecule was inserted into an expression cassette of the plasmid expression vector at a restriction site which in effect resulted in several additional nucleotides being added to the 5' end of the ultimate transcript. The predicted transcript which includes the dsRNA hairpin actually contains additional 5' and 3' sequences: a 5' leader consisting of 6 bases (e.g., the Sal I or Hind III or other chosen recognition sequence), followed by the dsRNA hairpin sequences, followed by a short 3' terminal U tract, usually two (1, 2, 3, or 4) U residues incorporated during transcription termination. These differences in length and composition of 5' and 3' transcript sequences flanking the dsRNA hairpin did not appear to adversely affect the ability of the dsRNA hairpin to effect dsRNA-mediated silencing, which suggests that, unlike synthetic dsRNA duplexes, endogenously expressed dsRNA hairpin constructs are effective despite varying in a number of respects, e.g., length of dsRNA "stem" between about 19-29 bp, length and composition of single-stranded loop, presence or absence of additional short 5' and/or 3' sequences.

A luciferase assay as taught in Example 1 of PCT/US05/29976 and in WO 04/076629, published 10-Sept-2004, "Methods and Constructs for Evaluation of RNAi Targets and Effector Molecules" indicated that all 4 promoter/shRNA cassettes were

active in silencing their target sequences in a cell supplied with the vector and an assayable substrate. The IC50 value decreased substantially when increasing from a one promoter/shRNA cassette vector to the pHB4 expression vector containing 4 promoter/shRNA cassettes. The IC50 values may have also been affected by the relative potency and transcription levels of each shRNA molecule, and did not reflect a simple relationship to the concentration of the vector only, which in effect behaved as four drugs after entering the cell and expressing the four encoded dsRNA molecules. In other words, the increased potency reflected not only the greater number of total shRNA transcripts generated by the vector, but the also the individual potency that each shRNA has to effect the reduction of sAg or eAg production via degradation of the target viral RNA molecules. The progressive addition of shRNA cassettes increased the potency of the vector in an apparently quantitative manner, and furthermore increased the pharmacological activity against the HBV target by inhibiting four distinct sites of the HBV target. It is important to recognize, however, that the ability of the multiple polymerase III expression constructs to express multiple individual antiviral dsRNA hairpin molecules is of significant value in and of itself, not just because of associated increases in "potency". Where the level of antiviral efficacy is high, the incremental quantitative increase in viral inhibition seen with each additional dsRNA molecule may be less important per se than the ability of the constructs to deliver what is in effect a multi-drug regimen, with the inherent advantage of being highly resistant to the development of viral resistance.

The expression vectors, designed to deliver multiple dsRNA effector molecules targeting highly conserved HBV and/or HCV polynucleotide sequences, when delivered to a virally infected cell, have the unique ability to destroy the viral nucleic acid products directly. Moreover, inherent and integral to the design and intent of these multiple promoter vectors (which express a plurality of different inhibitory short hairpin dsRNAs targeting different portions of the viral genome), is the property of generating multiple different viral antagonists simultaneously. The antagonists (short hairpin dsRNA effector molecules) target different genome sequences in the viral genome. One of these antagonists would probably be sufficient to disable the virus; however, the redundancy serves as a "backup" mechanism such that if the viral sequence mutates to render one antagonist inert,

there are 2, 3, 4 or more additional antagonists available. Additionally, by targeting multiple sites in the viral genome, different DNA or RNA products of the virus which play different roles in the disease pathology can be attacked at the same time.

In the case of Hepatitis B for example, in one embodiment, the instant invention uses 4, 5, or more shRNA molecules selected from the following sequences and other highly conserved HBV sequences as taught herein: e.g., "799" (SEQ ID No. 49); "1907" (SEQ ID No. 19); "2791" (SEQ ID No. 23); "1737" (SEQ ID No. 18), "1991" (SEQ ID No. 22), "1943" (SEQ ID No. 21). Other of the conserved HBV sequences disclosed herein, including sequences of e.g.,19 to 29 nucleotides, which comprise all or part of "799", "1907", "2791", "1737", "1991", or "1943", may be selected for inclusion in dsRNA hairpin effector molecules to be expressed by expression vectors comprising multiple promoters, including multiple polymerase III expression vectors. Due to the nature of HBV gene expression and overlapping transcriptional products this allows targeting of multiple RNA transcripts as well as the replicative template of the virus which will interfere with replication and expression of more than one of the viral proteins. One of the shRNA molecules, "1737" (SEQ ID No. 18) uniquely can disable the RNA encoding a product known as the X protein (HbX). Strong evidence exists in the biomedical literature that the X protein plays a role in establishment and/or maintenance of liver cancer. Because the existing drugs that can to some extent inhibit viral replication cannot eliminate the cell of integrated or other residual copies or portions of the viral genome, these drugs cannot shut off the production of HbX, even in patients "cured" of infectious HBV, and thus can not directly reduce any incidence of cancer that is mediated by dormant HbX. Multiple anti-HBV dsRNA hairpin expression constructs of the present invention can attack both the replication of the virus and the expression of all viral proteins, some which cause the inflammatory insult which results in hepatitis, and others such as HbX, which are believed to promote hepatocellular carcinoma via a distinct but not fully understood mechanism. It is recognized that the principles taught herein can be used to design constructs of the invention specifically tailored to treat such "post infection" patients, which express dsRNAs against Hbx and any other residual HBV antigens.

While the HBV target sequences of the invention were chosen expressly to represent those highly conserved or identical among a large number of different

isolates (strains) of HBV, for reference purposes the identified sequences, e.g., shRNA sequences, can be mapped back to HBV isolate AYW . It should be recognized, therefore, that the dsRNAs and dsRNA expression constructs of the invention are expected to be effective not only against HBV AYW and related viral strains, but against nearly all if not all HBV strains encountered in infected human populations in widely dispersed geographical areas.

Add, before the paragraph beginning at page 69, line 7, the following new section:

Several short duplex dsRNAs comprising the HCV sequences identified above in Table 14 (in each case, the first 21 bases constitute conserved HCV sequences of the invention, followed by an 8-base "adapter" sequence, "CCTGTCTC", appended from the Ambion kit used in synthesis, but which do not appear in the dsRNA effector molecules) targeting the 3'UTR; siRNA #12 targeting the HCV NS5B gene (positive control); the identified HCV core siRNA (positive control); and the identified lamin siRNA (negative control) were synthesized using the Silencer siRNA construction kit, Catalog # 1620 (Ambion Inc., Austin, Tex.). DNA oligonucleotides were synthesized by IDT (Coralville, Iowa).

Table 14A

siRNA number	SEQ ID NO	HCV sequence
#12	63	GCTAAACACTCCAGGCCAATA
#22	64	TCCTTTGGTGGCTCCATCTTA
#32	65	GCTCCATCTTAGCCCTAGTCA
#42	66	TCTTAGCCCTAGTCACGGCTA
#52	67	CCTAGTCACGGCTAGCTGTGA
#62	68	CTAGTCACGGCTAGCTGTGAA
#72	69	CGTGAGCCGCTTGACTGCAGA
#82	70	GCTGATACTGGCCTCTCTGCA
#102	71	ACTGGCCTCTCTGCAGATCAA

Replace the paragraph beginning at page 86, line 21, with the following amended paragraph:

This sequence is followed by a loop structure of 9 Ts. The second side of the hairpin contains a sequence that is complementary to the first side of the hairpin. One skilled in the art can easily design and construct hairpin constructs. Note: it is anticipated that other types of eiRNA vectors driven by other promoters, including RNA polymerase III promoters, and encoding other types of RNA structures, including various hairpin structures will have similar effects. Especially desirable are, e.g., "forced" hairpin constructs, partial hairpins capable of being extended by RNA-dependent RNA polymerase to form dsRNA hairpins, as taught in USSN 60/399,998P, filed 31-Jul-2002 and PCT/US2003/024028, filed 31-Jul-2003, as well as the "udderly" structured hairpins (e.g., multi-hairpin long dsRNA vectors and multi-short hairpin structures), hairpins with mismatched regions, and multiepitope constructs as taught in USSN 60/419,532, filed 18-Oct-2002, and PCT/US2003/033466, filed 20-Oct-2003, as well as a variety of other dsRNA structures known to those of skill in the art.

Add, before the paragraph beginning at page 87, line 14, the following new section:

EXAMPLE #7

Silencing HBV replication and expression in a replication competent cell culture model (see Example 1) using combinations of HBV- specific eiRNAs in multiple promoter vectors

As disclosed in PCT/US05/29976, filed 23-August-2005 and in U.S. Provisional Applications entitled "Multiple RNA Pol III Promoter Expression Constructs" (Serial No. 60/603622, filed 8/23/04, and Serial No. 60/629942, filed 11/22/04) the teaching of which is hereby incorporated by reference, two or more (preferably 3, 4, 5, 6 or more) of the shRNA sequences shown in Table I and SEQ ID NO:49 may be encoded in the same plasmid vector in separate cistrons under the control of separate promoters for each shRNA. SEQ ID NO:49 is:

GCCTCGCAGACGAAGGTCTCAAGAGAACTTTGAGACCTTCGTCTGCGAGGC

SEQ ID NO:49 represents the coding strand of a DNA sequence which encodes an shRNA molecule that targets an HBV conserved region. The first 21 bases of the

sequence above are identical to the sense sequence of HBV mRNA from position 799 to 779 in the HBV genome, strain AYW (numbered according to the complement strand given in Genbank Accession No. V01460). This sequence is followed by 9 bases (i.e., AGAGAACTT)representing the loop portion of the shRNA, followed by 21 bases of the reverse complementary sequence to the first 21 bases. (It will be understood that the loop sequence serves only to join the complementary sequences which form the double-stranded "stem" and therefore considerable variation in length and nucleotide sequence is acceptable within the loop region.) In a preferred embodiment, this DNA sequence will be placed in an appropriate expression vector operably under the control of a promoter, preferably an RNA polymerase III promoter such 7SK, U6, etc. The resulting RNA transcript:

GCCUCGCAGACGAAGGUCUCAAGAGAACUUUGAGACCUUCGUCUGCGAGGC

will assume a hairpin or stem-loop structure having 21 basepairs in a double-stranded conformation.

Using methods commonly employed by one skilled in the art of molecular biology, a single vector encoding two or more, preferably three or more, more preferably four or more, five or more, or all of SEQ ID NO:49, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23 is constructed. A particularly preferred embodiment for pharmaceutical applications of dsRNA-mediated silencing of the HBV target comprises a single expression construct encoding under the control of separate RNA polymerase III promoters, shRNAs corresponding to at least SEQ ID NO:19, SEQ ID NO:23, and SEQ ID NO:18, and optionally, SEQ ID NO:49 and/or SEQ ID NO:21. Such shRNA-expression vectors may advantageously utilize one or more RNA polymerase III promoters, including U6, 7SK, and H1 promoters in several alternative orientations and combinations. Particularly preferred constructs will utilize one or more of the 7SK promoters as taught in U.S. Provisional Serial Nos. 60/603622 and 60/629942. The instant example is thus analogous to Experiment 1 in Example 1 except that instead of introducing one vector with one shRNA at a time, the applicants deliver a single plasmid construct which expresses multiple shRNAs.

The advantages of this approach for therapeutic applications of dsRNA silencing are principally in the economy, simplicity and coordinated delivery of a single drug entity which comprises multiple different shRNAs each targeting a different site of the HBV genome. The ability to simultaneously target multiple sites of a viral genome is highly advantageous in preventing the clinically widespread phenomenon of drug resistance (by viral mutation), and the ability to combine dsRNA drug entities against these different target sites in a single delivery agent (the plasmid vectors of this invention) makes this conceptual approach uniquely feasible. While shRNAs, e.g., RNAs corresponding to SEQ ID NO:49, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23 may be produced, e.g., through chemical synthesis or *in vitro* expression, and delivered into an animal cell singly and in combination, there are significant advantages in some applications to express within the animal cell multiple shRNAs from a single expression vector. In this example, the potency of the multiple shRNA expression vectors significantly exceeded that of any one of the single vectors used in Experiment 1, as measured by similar assays.

EXAMPLE #8

Inhibition of Infectious Virions of HCV by dsRNA Effector Molecules

As a further example of HCV-targeted dsRNAs, the sequences given in Table 16 represent highly conserved coding region sequitopes from the 5' and 3' untranslated regions of HCV. Each sequence is written as the coding strand and is used to specify the design of a short hairpin dsRNA effector molecule comprising the coding sequence as shown in Table 16 connected to its reverse complement by a loop or linker sequence as described elsewhere herein. The sequences shown are predicted to be particularly efficacious as antiviral therapeutic agents because they were tested in a newly available *in vitro* HCV replication system capable of producing whole, infectious virions (disclosed in Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ., "Production of infectious hepatitis C virus in tissue culture from a cloned viral genome", Nat Med 2005 Jul;11 (7):791-6; and in Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV., "Robust hepatitis C virus infection in vitro", Proc Natl Acad

Sci USA 2005 Jun 28;102(26):9294-9). In this system, all viral proteins and viral nucleic acid sequences are present in a cell, as in a natural infection. In less complete replicon systems, dsRNA silencing molecules cannot be as rigorously tested as in the new system. It is expected that one or more (2, 3, 4, 5, or more) of the HCV sequitopes and their complements could be utilized as duplex dsRNA effector molecules, short hairpin dsRNA effector molecules, and/or encoded into dsRNA expression vectors capable of expression in vivo in a mammalian cell, including a human cell or organism.

Table 16

Seq Name	SEQ ID NO	Sequence (5' to 3')
HCV5M-5.1	72	AAAGGCCTTGTGGTACTGCCT
HCV5M-5.3	73	TTGTGGTACTGCCTGATAGGG
HCVXM-13	74	TAGCTGTGAAAGGTCCGTGAG
HCVXM-34	75	ATCTTAGCCCTAGTCACGGCTAGCTG
HCVXM-35	76	TAGTCACGGCTAGCTGTGAAAGGTCCG

The sequences in Table 17 represent additional preferred highly conserved at least 19 contiguous base pair HCV sequences from the 5' UTR of the virus (SEQ ID NO: 11). To generate the dsRNA effector molecules of the invention, these sequences are used in conjunction with their reverse complement and, optionally, a loop or linker sequence joining the sequence to its reverse complement, when it is desired to form a hairpin dsRNA effector molecule. One or more double-stranded RNA molecules comprising said conserved 5' UTR sequences (from SEQ ID NO: 11) may advantageously be used in combination with one or more other dsRNA effector molecules of the invention, including e.g., one or more highly conserved sequences from the 3' UTR (SEQ ID NO:27) and/or one or more at least 19 contiguous base pair sequences from SEQ ID NO. 12.

Table 17

HCV 5' UTR siRNAs		
Sequence Name	Sequence (5' to 3')	SEQ ID NO
HCV5P-1.1	CCTGTGAGGAACTACTGTCTT	77
HCV5P-1.2	ACGCAGAAAGCGTCTAGCCAT	78
HCV5P-1.3	CGTCTAGCCATGGCGTTAGTA	79
HCV5P-1.4	GTCTAGCCATGGCGTTAGTAT	80
HCV5P-1.5	CTCCCCTGTGAGGAACTACTGTCTT	81
HCV5P-1.6	GAGGAACTACTGTCTTCACGCAGAA	82
HCV5P-1.7	GTGAGGAACTACTGTCTTCACGCAGAA	83
HCV5P-2.1	GAGCCATAGTGGTCTGCGGAA	84
HCV5P-2.2	GAACCGGTGAGTACACCGGAA	85
HCV5P-2.3	ACCGGTGAGTACACCGGAATT	86
HCV5P-2.4	GGGAGAGCCATAGTGGTCTGCGGAA	87
HCV5P-5.1	GGCCTTGTGGTACTGCCTGAT	88
HCV5P-5.2	GCCTTGTGGTACTGCCTGATA	89
HCV5P-5.3	GTACTGCCTGATAGGGTGCTT	90
HCV5P-5.4	AAGGCCTTGTGGTACTGCCTGATAGGG	91
HCV5P-5.5	CGAAAGGCCTTGTGGTACTGCCTGATA	92
HCV5P-5.6	CTTGCGAGTGCCCCGGGAGGTCTCGTA	93
HCV5M-1.1	ATCACTCCCCTGTGAGGAACT	94
HCV5M-1.2	TTCACGCAGAAAGCGTCTAGC	95
HCV5M-1.3	TAGCCATGGCGTTAGTATGAG	96
HCV5M-1.4	ATCACTCCCCTGTGAGGAACTACTG	97
HCV5M-1.5	ATCACTCCCCTGTGAGGAACTACTGTC	98
HCV5M-1.6	AACTACTGTCTTCACGCAGAAAGCG	99
HCV5M-1.7	AACTACTGTCTTCACGCAGAAAGCGTC	100
HCV5M-2.1	ATAGTGGTCTGCGGAACCGGT	101
HCV5M-2.2	TAGTGGTCTGCGGAACCGGTG	102
HCV5M-2.3	AACCGGTGAGTACACCGGAATTGCC	103
HCV5M-5.2	AAGGCCTTGTGGTACTGCCTG	104
HCV5M-5.4	TACTGCCTGATAGGGTGCTTG	105
HCV5M-5.5	TTGTGGTACTGCCTGATAGGGTGCTTG	106
HCV5M-5.6	TACTGCCTGATAGGGTGCTTGCGAG	107
HCV5M-5.7	TAGGGTGCTTGCGAGTGCCCCGGG	108
HCV5M-5.8	TTGCGAGTGCCCCGGGAGGTCTCGTAG	109

Please replace the Sequence Listing with the attached Sequence Listing.